NIDDK Recent Advances & Emerging Opportunities

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National Institute of
Diabetes & Digestive &
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CONTENTS

Message from the Director

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) continues to support a strong portfolio of basic and clinical research, encompassing a wide range of chronic and costly diseases. With NIDDK funding, talented researchers are capitalizing on the wealth of new knowledge generated by the biotechnology revolution, genetics research, cell biology and other fields. Building on this progress, we now have unprecedented opportunities to combat many critical health issues, including diabetes, gastrointestinal problems, diseases of the kidney and urinary tract, and blood disorders. Finding safe and effective treatments and developing strategies to both cure and prevent disease are the ultimate endpoints toward which we strive.

We recognize that this year has been particularly challenging for America. No one—no matter what his or her field of endeavor—has been untouched by the tragedy of September 11, 2001, and its aftermath. With the unbearable sadness of this tragedy has also come a renewed dedication on the part of all Americans to work together toward our mutual goals. Biomedical research well exemplifies this commitment, as its goal is to improve and enhance the health and well-being of all Americans through the discovery and application of new scientific knowledge. The terrible events of September 11 have served to remind us that life is indeed a precious gift. The vigorous biomedical research efforts of the NIH are a critical means of preserving and extending this gift by bringing the benefits of American science to the world.

A few examples of the many research advances by NIDDK-funded scientists are provided in this annual compendium, along with highlights of the technologies that made these achievements possible. These advances range from basic findings in genetics—such as discovery of a novel susceptibility gene for Crohn's Disease—to impressive results from clinical trials—such as the demonstration that type 2 diabetes can be prevented or delayed in a diverse American population. These are just two examples from the many research advances presented in this booklet, most of which were published in fiscal year 2001. In addition, we have also included a few "Stories of Discovery," which trace research advances over a much longer period of time and illustrate how the accumulation of new knowledge is often an incremental process, with each new finding adding to the science base from which clinical advances eventually flow. Also featured are some personal stories of patients whose lives have been adversely affected by disease, and for whom research brings hope.

We view this publication as a vignette of the impressive accomplishments of NIDDK-supported researchers, as well as the enormous promise their research efforts hold for the future. The examples given here are representative of the much larger and broader research portfolio funded by the NIDDK. In developing this booklet, we have simply tried to give a sampling of the vigorous research efforts we are supporting across the many scientific disciplines and categorical disease areas encompassed within the NIDDK mission. As we mine these research advances for further insights and new opportunities, we remain committed to the translation of science into improvements in the quality of life for all people. We are dedicated to that goal.

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Cross-Cutting Science: Paving the Way to Discovery

Advances in medicine are largely dependent on the accumulation of new knowledge about biologic processes, especially at the smallest levels of an organism–its genes, the proteins they control, and the workings of cells. While the ultimate application of such basic research is not always obvious to the public, major strides in fighting disease can be traced back to laboratory studies whose immediate relevance to health could not have been fully known or appreciated at the time they were conducted. Described here are some recent studies of fundamental processes, as well as the technologies that make such studies possible. The insights gained through this type of research can be expected to propel disease-oriented research, not only within the NIDDK mission, but also in many other fields. Investment in such cross-cutting scientific research today will have future applications that we cannot now describe with certainty, but which we know will surely be realized.

Functional Genomics: Tools for Discovering the Functions of Genes Relevant to Disease

In the quest for new and better treatments for disease, biomedical scientists are creating and using exciting advances in modern technology to discover important genes and learn their functions at an ever-increasing rate. This knowledge can propel new advances in diagnostics and drug development. Because different diseases and medical conditions arise from disparate causes—mutations in genes, infectious agents, and even adverse reactions to certain medical therapies—scientists delve into the inner workings of living cells with a great diversity of approaches. In genetic research, genes are often identified, and clues to their function obtained, by investigating what goes wrong when a gene is mutated, as is the case in many diseases. The study of genes has been revolutionized by modern functional genomics, the use of large-scale, high-throughput techniques to discover the function of genes and how all the genes in the genome of an organism work together. For example, scientists can now scan hundreds or thousands of genes at a time to see which may be active in a certain type of cell. For cases in which a disease is caused by a mutation in a gene that is already known from previous research, new technologies are also expediting experiments in which scientists generate an animal with an analogous mutation. From the animal model of the disease, they can learn how the disease progresses and what other genes may be involved, and they can use these animal models to test candidate therapies that are not yet ready for human trials. Because most genes are blueprints for the construction of specific proteins, scientists also gain critical insights into disease by studying how proteins function—or malfunction—when genes are mutated. In parallel with investigations into the causes of particular diseases, researchers also seek to build upon fundamental knowledge of genes and their functions to perpetuate the cycle of scientific discovery: critical insights and breakthroughs in medicine are often predicated upon the accumulation of such knowledge and upon the development of new technologies. Thus, investment in genetic and genomic research can be expected to have cross-cutting implications, advancing medical research within the NIDDK mission and in other fields as well.

Modeling the Control of Cell Growth Through Mice: Cancer is the result of uncontrolled cell growth. Normally, the body regulates when a cell should grow and divide to produce new cells where they are needed, and when the growth should stop. A cell must also carefully monitor its genome as it grows to ensure that if mutations arise, they are not propagated into new cells. How this elaborate regulatory system breaks down to allow mutant cells to grow into tumors has been the subject of much research. Mouse models of cancer are particularly useful to researchers precisely because they provide insights into the development of cancer in the context of a whole

organism, where all of the regulatory systems should normally be in place.

Cell Growth and DNA Damage in Breast Cancer: Breast cancer strikes approximately one in nine women. It can be caused by new mutations or by mutations that were inherited. Many of the hereditary cases of breast cancer are caused by mutations in the gene *BRCA1*. *BRCA1* appears to be a central player in many biological pathways, including regulating cell growth and maintaining the integrity of the genome through repair of DNA damage. Thus, mutations in this gene are particularly insidious because without *BRCA1*, cells are left vulnerable to acquiring even more mutations.

While some mutations are caused by environmental factors, other mutations just occur spontaneously in cells. One of the body's defenses against DNA damage is to have cells die rather than perpetuate potentially harmful mutations; this programmed cell death is called "apoptosis." How do some *BRCA1* mutant cells escape death and instead grow uncontrollably into tumors? Scientists recently deduced that some of these cells do so by mutating the protein that imposes the death sentence: this protein is called p53.

In studies in mice, NIDDK-funded scientists found that *BRCA1* mutations resulted in massive numbers of dead cells as a result of apoptosis. This was likely in response to DNA damage, the result of the accumulation of many spontaneously arising mutations left unchecked in the absence of *BRCA1*. Surprisingly, this massive cell death was not seen in mice that both lacked *BRCA1* and additionally had a mutation that impaired p53 function—and most of these mice eventually developed breast tumors. These results are particularly significant to human cancer because *BRCA1*-associated tumors have a relatively high frequency of p53 mutations.

Mutations in p53 have long been associated with many types of human cancers. One possible reason for this is that p53 can suppress uncontrolled cell growth in several ways, including by triggering cell death. To further investigate the interactions between p53 and *BRCA1*, the researchers induced extensive DNA damage in mice by exposing them to radiation. The cells of normal mice bolstered their levels of p53 protein to help protect against the damaging effects of the radiation, but in mice with *BRCA1* mutations, the p53 response was impaired. These results help show that in normal cells, *BRCA1* and p53 must coordinate to protect against conditions that can lead to cancer.

These experiments suggest a mechanism for *BRCA1*-mediated tumor formation. In the absence of normal *BRCA1* function, mutations accumulate in the DNA. In some cells, this DNA damage eventually strikes the gene encoding the p53 protein, causing a mutation that destroys it. Once p53 function is lost, these mutant cells can escape death, continue to grow and divide, and eventually form tumors. The mice developed in this study, with *BRCA1* and p53 mutations, will continue to be of value to increase our understanding of cancer progression; these mice may also serve as useful models to test new treatments for breast cancer.

Multiple Endocrine Neoplasia: Multiple endocrine neoplasia is a cancer syndrome characterized by multiple tumors in the parathyroid glands, pancreas, anterior pituitary, and other parts of the body. It is caused by mutations in a gene called *MEN1* (for multiple endocrine neoplasia type 1), which was discovered by the collaborative efforts of NIH scientists from the NIDDK, the National Cancer Institute, and the National Human Genome Research Institute. To gain further insight into this cancer syndrome, scientists recently generated a mouse model of the disease. Like humans, mice have two copies of the *MEN1* gene. Using genetic engineering, the researchers mutated one of these copies. The mice developed symptoms remarkably similar to human multiple endocrine neoplasia, including tumors in the pancreas, parathyroid, pituitary, and other tissues. The scientists observed that the tumor cells had spontaneously lost the remaining normal copy of the *MEN1* gene. Future research on these mice may reveal whether other genetic events accompany tumor formation. Ultimately, as current therapies for this syndrome are often unsatisfactory, the availability of a mouse model should be an asset for the testing of possible new therapeutic approaches.

Unfolding Protein Folding: Just as gears and wires must be perfectly crafted to make a machine work, the proteins of the human body must assume very distinct shapes to perform the functions necessary for life. A gear of the wrong shape or a mass of wires tangled randomly together will disrupt a machine's function. Likewise, an improperly-shaped protein, or certain aggregates of proteins interacting abnormally, can lead to devastating diseases such as Alzheimer's and "mad cow" disease. Proteins are chains of amino acid building blocks; the

nature and order of amino acids in each protein are dictated by the sequence of the gene encoding the protein. Each amino acid chain must "fold" into a particular intricate shape so that the protein can function. A mutation in a gene that changes the nature of even just one of the amino acids can make it impossible for the protein to fold properly. A few types of proteins seem to be able to change their structure spontaneously—more alarmingly, some of these proteins sabotage the folding of other proteins, even in the absence of genetic mutations. Proteins that can do this after infecting a living organism are called prions. Research on prions and on abnormal aggregates of misshaped proteins, called amyloids, continues to shed light on protein-folding diseases and will lead to new ideas for treatment approaches.

Prions and Amyloids in a Yeast Model System: Many diseases that ravage the brain, such as the much-feared mad cow disease and other transmissible spongiform encephalopathies, are caused by infectious proteins called prions. In recent years, creative experiments by NIDDK-funded scientists in a seemingly unlikely model system—baker's yeast—have provided insights not only into prion formation, but also into amyloids, which are abnormal forms of protein observed in many diseases.

Yeast are ideal model organisms for investigating many biological processes because they are readily amenable to highly sophisticated genetic manipulation and other experimental techniques; they require little storage and growth space; and they are relatively inexpensive to grow. (Such features would contrast sharply, for example, with a herd of "mad cows" that one might wish to study.) Yeast also have their own set of prions which, like those of animals and people, often start out as normal proteins but then spontaneously change into a sinister form. Prions propagate by converting other proteins into this abnormal form. One such yeast prion is called [URE3], which is an altered form of a normal yeast protein called Ure2p.

Scientists recently learned that yeast prions such as [URE3] do not propagate by themselves: they use other proteins, called chaperones, as unwitting accomplices to help them convert more Ure2p proteins into [URE3] prions. Further experiments showed that [URE3] prions aggregate together to form networks of amyloid filaments, which resemble amyloid found in a number of human diseases, including Alzheimer's, late-onset diabetes, multiple myeloma, and transmissible spongiform encephalopathies. Continued research on yeast prions will generate further insights into both prion disease and amyloid formation and propagation.

Protein Folding in Amyloid Disease—Implications for Therapy: A recent study of the disease familial amyloid polyneuropathy explained an intriguing case of two genetic wrongs making a right. The disease is caused by a mutant version of the protein transthyretin. People have two copies of the gene for transthyretin, one from each parent. If one copy is normal, but the other copy codes for a protein with a mutation called "V30M," then disease occurs. (V30M is a shorthand designation that scientists use to note that the 30th amino acid building block of the protein is mutant. The chemical nature of the defect is abbreviated by the letters V and M.) Curiously, individuals are protected from disease if the second copy of the transthyretin gene—rather than being normal—has a different mutation, called "T119M."

NIDDK-funded scientists recently discovered how the T119M mutation overcomes the adverse effects of the V30M mutant. Transthyretin proteins usually snap together in groups of four, but the V30M mutation renders them unable to maintain this normal configuration. Separated from the group, the individual proteins with the V30M mutation begin to unfold, lose their characteristic shape, and then aggregate in a harmful mass that interferes with nerve and muscle function. By contrast, scientists found that proteins with the T119M mutation exert an especially stabilizing influence on the transthyretin group, even if the foursome includes some of the V30M mutants. (Not all mutations, then, are bad.) The extra stability of the T119M version of transthyretin counteracts the unfolding propensity of the proteins carrying the V30M mutation. The implications of this finding are that this amyloid disease—and potentially others like it, such as Alzheimer's—may be amenable to treatment strategies designed to stabilize the proper groups of proteins to prevent misfolding.

"Insights" into the Digestive System—Feeding Fluorescent Fats to Zebrafish: In a clever new approach to identify genes involved in fat processing, scientists combined modern genetic techniques with glow-in-the-dark fats. The model organisms they used, zebrafish larvae, process fats in the intestine and liver and respond to cholesterol-blocking drugs in a manner similar to humans. Thus, genes identified as important in zebra-

fish for fat processing are likely to be important in humans also.

Zebrafish are common pets throughout the world. In recent years, however, they have acquired a new purpose: they have become established as a powerful model organism for biological research. Zebrafish are readily amenable to genetic manipulation, facilitating the identification and characterization of genes. They are vertebrates, with organ systems similar to those of people and other mammals. Because zebrafish are relatively small, scientists can maintain large numbers of them in the lab. Zebrafish also have a striking characteristic that makes them particularly valuable for studying how internal organs and tissues are formed: during their rapid development, the fish embryos and larvae are transparent, permitting their insides to be viewed easily. Thus, the zebrafish can be a virtual window to enable researchers to see how cells differentiate and organs develop.

Exploiting the ability to synthesize fluorescent fats and the ability to perform sophisticated experiments on zebrafish, NIDDK-funded scientists screened for and identified zebrafish larvae with mutations that disrupt proper fat processing. After these mutant larvae were fed fluorescent fats, their gall bladders did not glow as brightly as those of normal larvae. Thus, the scientists can deduce that the genes that were mutated must be important for fat processing. By tracking down the location of the mutations within the genome, the researchers will eventually be able to find and study these important genes.

One of the mutants, nick-named "fat-free," had a digestive tract that otherwise appeared normal. Thus, without the use of fluorescent fat technology to detect a defect, this mutant would have been overlooked, and potentially valuable insights into the genetics of the digestive system would have been missed. This research predicts that genetic screens in zebrafish, along with sensitive fluorescent fat technology, may identify genes important for diseases of fat metabolism, biliary disorders, and even certain types of cancer in which fat signaling plays a role.

With support from multiple NIH components under the leadership of the NIDDK and the National Institute of Child Health and Human Development, the community of scientists who study zebrafish have been developing sophisticated genomics tools to facilitate the mapping and identification of important genes and to determine their functions. An effort to sequence the entire zebrafish genome is now beginning.

Using a Genetic Database to Prevent Blood Transfusion Reactions: Blood transfusions save the lives of accident victims, surgery patients, and people suffering from blood disorders such as dialysis-induced anemia, sickle cell anemia, and Cooley's anemia. However, patients can develop an adverse reaction to transfused blood if certain molecules displayed on the surface of the donor red blood cells differ from those on the patient's own cells. There are many groups of such surface molecules, including the group used to classify blood into the commonly-known A, B, or O types. Among the many other blood groups is one called the Dombrock group, named after a blood donor named Dombrock in 1965. A reaction against a surface molecule of the Dombrock group can cause the destruction of transfused blood cells as well as fever, chills, and other symptoms. However, reliable products have not been available to screen blood for the Dombrock type.

Recently, with modern large-scale genomic techniques, NIDDK-funded investigators working at the NIH discovered the gene coding for the Dombrock molecules. They began with two clues from prior research. First, genetic studies had linked the Dombrock gene to chromosome 12. Second, research on red blood cells suggested that the Dombrock molecules are anchored to the cell membrane in a specific way. The scientists prepared DNA from developing red blood cells (because mature red blood cells lack chromosomes) and generated a database of 5,000 genes that are active in these cells. They then screened this database to look for a gene that both localized to chromosome 12 and that also had a sequence characteristic of molecules that are anchored to cell membranes in the same way as the Dombrock molecules.

The strategy worked. Over thirty years after the initial identification of Dombrock blood types, the gene that encodes the Dombrock molecules has now been cloned. The scientists were even able to find versions of the gene with slight sequence variations that correlate with the different types of Dombrock molecules. With this breakthrough, gene-based methods can now be developed to screen patients, and also donor blood, for Dombrock type. This type of screen would allow doctors to match a patient with donor blood of the same Dombrock type to reduce painful transfusion reactions.

This research also illustrates the value of a database of genes that function in red blood cells. Such a database will undoubtedly propel further discoveries in red blood

cell biology in order to better understand and treat human diseases involving these cells. With this as a goal, NIDDK is supporting the development of a large database, called "Hembase," to provide worldwide access to genetic-based studies of red blood cells performed by scientists working at the NIH.

Taking Tools into the Future—Building Knowledge of Disease Genes: The NIDDK is continuing to advance biomedical research by identifying and pursuing cross-cutting areas of research within its mission that can be addressed with recent and developing technologies.

Modifier genes: The symptoms and severity of all diseases vary from one person to the next due to differences in genetic makeup and environmental exposure. For many medical disorders, a mutation in a single gene plays the predominant role in the development of disease. However, even among those who have identical mutations in such a gene, the severity of the disease can vary. To better understand this phenomenon, it is necessary to find other genes, called "modifier genes," that contribute to this variability. Identifying these modifier genes would improve our ability to predict the symptoms and severity of a disease in a particular individual, and may lead to improved treatments. With the primary genes for many diseases now known, the NIDDK will strive to build upon our understanding of the genetics of these diseases by stimulating research to identify modifier genes. A planned symposium on the search for modifier genes will encompass such diseases as cystic fibrosis, polycystic kidney disease, Gaucher disease, and other disorders within the NIDDK mission.

Increasing Understanding of Membrane Transport: Cell membranes are like the walls of a house, with windows and doors that can open to the outside and to interior rooms. On a much smaller but far more elaborate scale, the membranes of living cells are designed to let nutrients and other molecules pass through in a highly-regulated fashion. Many diseases, such as cystic fibrosis, diabetes, renal tubular acidosis, congestive heart failure, and several intestinal disorders, arise from defects in the transport of substances across membranes. Membrane transport processes in humans and other mammals are very similar to those in lower organisms, such as bacteria and yeast, among others. These similarities can be exploited to build upon our knowledge of membrane transport because these non-mammalian organisms are also easily experimentally-manipulated (more so than mammals), and the genome sequences for many of these are also known. The NIDDK plans to support innovative approaches to studying membrane transport in bacteria, yeast, zebrafish, and other non-mammalian organisms, and to search for novel genes and discover how they function. This knowledge will lead to important insights into the workings of human cells and membrane transport-associated diseases.

Beta Cell Biology Consortium: Central to the onset and progression of diabetes are abnormalities in cells called beta cells. Beta cells reside in the pancreas and produce insulin, a hormone that is essential for life. In type 1 diabetes, beta cells are destroyed, so no more insulin is produced. In type 2 diabetes, the body does not respond properly to insulin; the beta cell initially compensates by secreting extra insulin but eventually fails, leading to overt diabetes. It is believed that signaling defects involving beta cells are at the root of insufficient insulin secretion in type 2 diabetes. A comprehensive understanding of beta cells will therefore lead to new diagnostic and treatment approaches for both forms of this devastating disease. The NIDDK has launched a functional genomics initiative to identify the genes that are critical for beta cell development and to learn how they function. To build on this initiative, the NIDDK proposes to establish a Beta Cell Biology Consortium. Through the Consortium, individual Beta Cell Biology Programs would have access to information, resources, technologies, expertise, and reagents that are beyond the scope of any single research effort. The goals of the Consortium will be to advance our knowledge of beta cell biology and to develop technologies that will have implications for early detection of disease, for therapeutic transplantation of beta cells in type 1 diabetes, and for understanding derangements of cell signaling and regulation in type 2 diabetes.

Progenitor Cell Genome Anatomy Projects (GAPs): The successful treatment of many chronic and debilitating diseases afflicting Americans today will depend on the ability to replace organs or to stimulate regeneration and recovery of damaged organs. To build upon the achievements of the Human Genome Project, the NIDDK and other NIH institutes have established a range of Genome Anatomy Projects (GAPs) to map the complex network of cellular interactions in normal and diseased tissues. In the NIDDK, for example, one new initiative is the Diabetes Genome Anatomy Project, which will look at the

expression of genes in pancreatic and other tissues affected by diabetes. Now, complementary new research initiatives will apply this same comprehensive approach to focus on progenitor cells. Progenitor cells develop into different types of cells that form the organs and tissues of the body. It is important to understand how tissues and organs develop from progenitor cells, and how progenitor cells maintain and regenerate tissues and organs in health and disease. Approaches will include developing biomarkers to detect and classify stem cells and progenitor cells; profiling the cells to catalogue genes that are active; and creating tools for characterizing the functions of these genes. Such research would not only capitalize on the sequence data from the Human Genome Project, but could also take full advantage of human embryonic stem cell lines which meet established criteria for use in research supported by the NIH. It is also a goal that well-characterized cells, DNA, and specific tools for progenitor cell analysis developed by the GAPs will be distributed to the broader research community. Further, the development of bioinformatics systems, including databases, will ensure that data produced are available to researchers worldwide soon after being generated in the laboratories.

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Stem Cells: Developing Potential

Scientists are striving to understand the processes that occur during normal development, when a vast number of different cell types are generated from a single fertilized egg. If they can understand normal development, scientists will have a better chance of determining how to recapitulate development in an adult in order to replace cells damaged by disease. Even within adults, special cells known as stem cells retain the ability to divide, and the divisions can give rise either to more stem cells or to cells that will differentiate into specific cell types. This process is analogous to harvested wheat seeds: the farmer can use the seeds either to plant more wheat, as when stem cells divide to produce more stem cells, or to produce specific products such as bread, as when stem cells divide to produce cells that differentiate into specific cell types.

Currently, scientists are determining the usefulness of different types of stem cells for treating human disease. Until now, replacement of cells has only been possible via organ or cell transplantation. Doctors are unable to treat every needy patient with transplantation, however, because there are limited supplies of donated cells and organs. Stem cells are heralded as a possible means for overcoming this treatment barrier.

The various types of stem cells are believed to differ mainly in the limits of their "potential"—their ability to produce other cell types. Embryonic stem cells arise early in development. Because they must give rise to all the different cell types and tissues of the body, embryonic stem cells are thought to have almost unlimited potential. Adult stem cells, on the other hand, reside within a mature tissue or organ and are thought to be able to differentiate into a more limited number of cell types. Experiments are still being performed to test the validity of these assumptions.

In the past year, NIDDK-funded scientists studying stem cells have made a number of exciting discoveries. Investigators identified a population of adult stem cells present in both rat and human pancreas capable of generating all types of pancreatic cells in culture. Investigators studying adult stem cells in the blood were surprised to discover that they are capable of producing not only numerous types of blood cells, but also liver, lung, gut, and skin cells. Another team showed that stem cells of the adult mouse pancreas are capable of producing both

pancreas and liver cells. Still another group of investigators developed a new technique for expanding cultured umbilical cord stem cells in order to generate enough cells for a transplant. These studies have all advanced our progress towards developing alternative sources of cells for transplantation to treat human diseases.

The NIDDK is supporting several efforts designed to capitalize on and extend previous stem cell and developmental biology discoveries and to stimulate new discoveries. As previously mentioned, two Genome Anatomy Projects (GAPs) support the use of advanced technologies and bioinformatic techniques to describe gene expression patterns in stem cells both during development, and in adult stem cells during tissue maintenance and tissue repair following disease. The planned Progenitor Cell GAPs will support research to identify and describe stem cells located within specific tissues of the gastrointestinal lining, liver, exocrine pancreas, kidney, urinary tract, prostate, and bladder. Hematopoietic Cell Lineage GAPs will support work to describe gene expression in blood (hematopoietic) stem cells. Another NIDDK effort will sponsor studies to describe normal development and stem cells of the gastrointestinal tract, liver, and exocrine pancreas. The NIDDK hopes that knowledge gained from these studies will enable doctors to use stem cells and developmentally-regulated genes to repair and replace damaged and diseased tissue.

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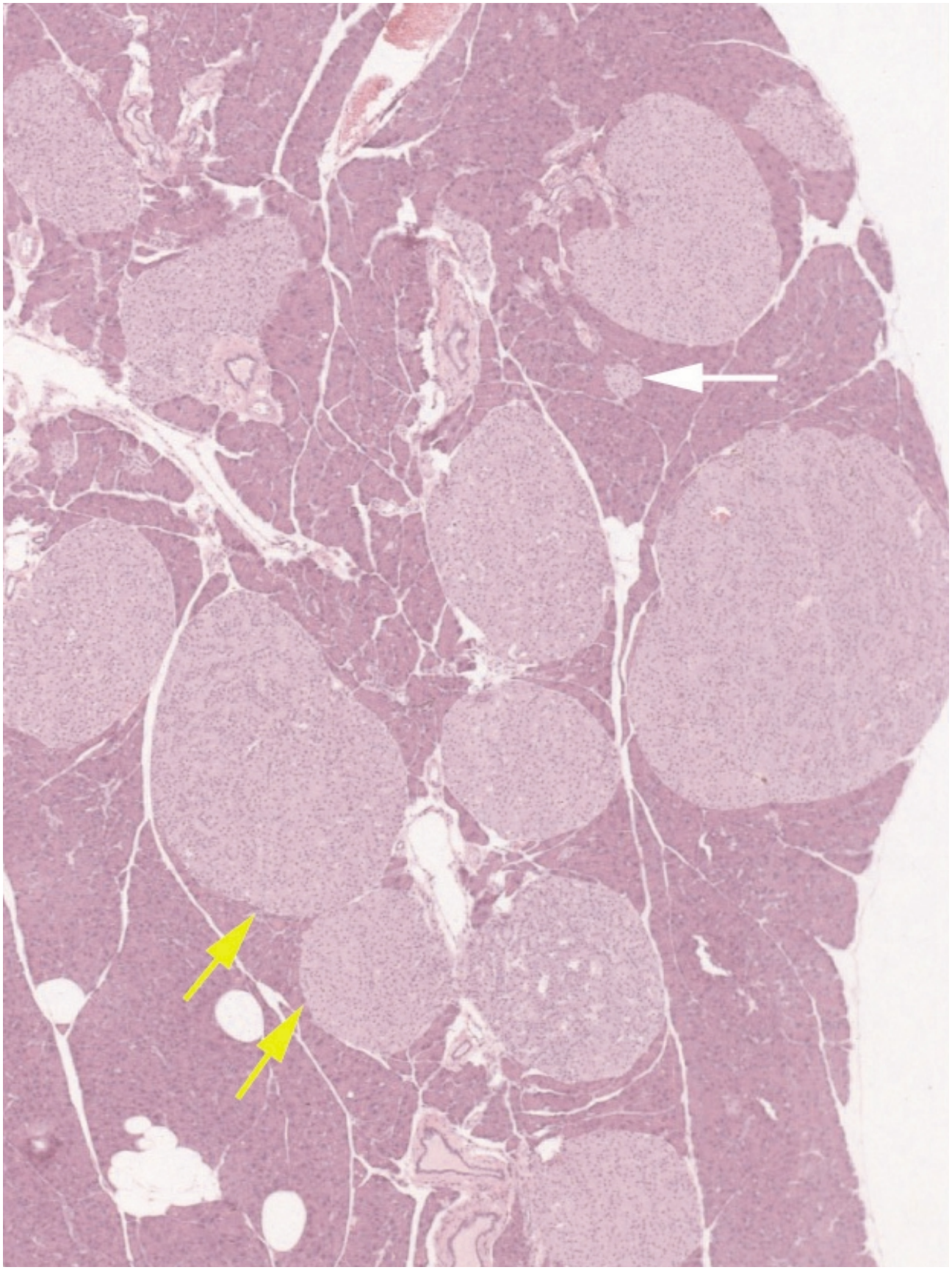

This photograph shows a stained tissue section from a mouse pancreas. Clusters of pancreatic cells called "islets" (pale purple) produce the hormone insulin, which is crucial for proper glucose metabolism. A normal insulin-producing islet is indicated by the white arrow. A mutation in one of the mouse's genes, similar to one found in the human disease multiple endocrine neoplasia, caused uncontrolled growth in a large number of the islets (yellow arrows), resulting in pancreatic islet tumors that over-produce insulin. Just as too little insulin can lead to diabetes, too much insulin can cause dangerous drops in blood sugar levels (hypoglycemia). Photo credit: Dr. Judy S. Crabtree, National Human Genome Research Institute, National Institutes of Health.

Diabetes, Endocrinology and Metabolic Diseases

Diabetes is one of the leading causes of disability and death in the U.S. It affects an estimated 16 million Americans, about one-third of whom do not even know they have the disease. The causes of diabetes are not precisely known, but both genetic and environmental factors play a role. Although there are several interventions currently available to help reduce the burden of this disease, there are no methods to cure it. The disease is marked by deficiencies in the body's ability to produce and properly use insulin—a hormone that is essential for the conversion of food-derived glucose into energy necessary for daily life. As a result, glucose becomes elevated in the blood, with detrimental effects on both small and large blood vessels. The most common forms of the disease are type 1 diabetes, in which insulin-producing capacity is totally destroyed, and type 2 diabetes, in which the body is resistant to insulin, even though some amount of insulin may be produced. Both forms of diabetes can lead to serious and costly complications, including kidney failure, blindness, amputations, heart disease and stroke. According to the American Diabetes Association, diabetes and its complications cost nearly $100 billion annually.

Type 1 diabetes most often occurs in children, but can appear at any age. Formerly known as insulin-dependent or juvenile-onset diabetes, it accounts for 5 to 10 percent of all diabetes in the U.S. It occurs equally among males and females, but is more common in Caucasians than in non-Caucasians. Type 1 diabetes develops when the body's system for fighting infection—the immune system—turns against itself in a disease process termed "autoimmunity." The immune system destroys clusters of cells in the pancreas called islets, which contain the body's insulin-producing beta cells. Once these cells are destroyed, type 1 diabetes patients require either lifelong insulin injections, often multiple times throughout the day, or infusion of insulin via a pump to control their blood glucose levels. Insulin therapy, however, is not a cure, nor can it always prevent the long-term complications of the disease.

Type 2 diabetes is the most common form of the disease. Once known as non-insulin-dependent or adult-onset diabetes, it affects about 90 to 95 percent of people with diabetes. Type 2 diabetes is more common in older people, especially older women who are overweight. Obesity is a major risk factor for this form of diabetes (see advances in obesity research on page 39). It also occurs more frequently among minority groups, including African Americans, Hispanic Americans, Native Americans, and Native Hawaiians. Recently, largely because of an increased incidence of childhood obesity, a disturbing increase of type 2 diabetes has been reported in children, particularly minority children. In patients with type 2 diabetes, cells in muscle, fat and liver tissue do not respond effectively to insulin. Gradually, the pancreas secretes less and less insulin in response to meals, and the timing of insulin secretion becomes abnormal. As clinically recognizable diabetes develops, production of insulin continues to decline. To control glucose levels, treatment approaches include diet, exercise and medications; some patients also need to take insulin. Type 2 diabetes is now approaching epidemic proportions in the U.S. The Centers for Disease Control and Prevention estimates that, if current trends continue, the number of people with diagnosed diabetes in the U.S. is expected to increase by 165 percent by the year 2050. Left untreated, the alarming increase of type 2 diabetes in children could cause these projections to burgeon even further.

Cell Signals and the Development of Type 2 Diabetes: Insights from Animal Models

As demonstrated by several recent advances, mouse models of diabetes have been pivotal in increasing our understanding of the pathways involved in the control of insulin secretion and the maintenance of normal, stable glucose levels in the blood. Such mouse models can help show us why the muscle, liver and fat cells of many individuals, especially those who are overweight or obese, lose the ability to respond to insulin effectively—thereby causing them to develop "insulin resistance." When this happens, the pancreatic beta cells must produce ever-increasing amounts of insulin to main-

tain normal blood sugar levels. For reasons that are poorly understood, the pancreas is unable to keep up with this increased demand in some individuals, and they progress from insulin resistance to the development of full-blown "type 2" diabetes.

Mechanisms of Insulin Resistance: Genetically engineered mice have been used by NIDDK-funded scientists to help unravel the mechanisms underlying the insulin resistance seen in type 2 diabetes, and its association with obesity. The mice were engineered to have mutations that would eliminate, or "knock out," the function of a protein, GLUT4, that transports glucose into cells. Recent technology enabled the scientists to remove GLUT4 only from fat cells, rendering them unresponsive to insulin-induced glucose uptake, but leaving the insulin signaling apparatus intact in other tissues such as muscle and liver. Surprisingly, the lack of GLUT4 in fat cells not only made these cells insulin-resistant, but also made the muscle and liver insulin-resistant. This finding suggests that fat cells normally secrete a factor that travels in the blood to the muscles and liver and that the absence of GLUT4 changes the amount of this factor circulating in the blood. After further experiments, researchers concluded that fat cells must use messengers to "talk" to liver and muscle cells. These results suggest that there may be a number of as yet undiscovered signaling molecules involved in insulin-induced glucose uptake that could prove to be useful drug targets in the treatment of type 2 diabetes and obesity.

Control of Glucose Production: In related research in mice, another team of investigators made a significant discovery about the cell-signaling machinery that controls glucose production by the liver. They found that low blood glucose signals tell liver cells to turn on the production of a known protein, PGC-1, which—in turn—interacts with other molecules to activate a series of genes required to produce glucose.

Normally, the body maintains exquisite control over levels of glucose in the blood. During periods of fasting, the liver manufactures glucose for cells, especially brain cells, to use as an energy source. After a meal, insulin signals the liver to turn down its glucose production. This process maintains blood glucose levels within a very narrow range. When insulin production is abnormally reduced or nearly abolished, as in type 2 or type 1 diabetes, respectively, the liver does not receive the signal to reduce glucose production. Instead, the liver continues to pump glucose into the bloodstream, and high blood glucose levels can lead to devastating complications.

In their studies, the NIDDK-funded researchers found that, whereas PGC-1 is present in the liver cells of normal mice only when glucose production is needed, diabetic mice appear to make PGC-1 continuously. Thus, PGC-1 functions as an "on-off switch" for glucose production by the liver. These results are the first demonstration that PGC-1 is important for glucose production by the liver, and have major implications for the treatment of diabetes. For example, the anti-diabetes drug, metformin, shuts down glucose production in the liver, but exactly how it does this is unknown. Future research based on the findings about PGC-1 could lead to insights into the mechanism of action of metformin and other anti-diabetes drugs, and may also aid in the development of new and more effective drugs for use in the treatment of type 2 diabetes.

Effects of the Appetite-Controlling Hormone Leptin: In yet another study of diabetes in mice, NIDDK scientists, in collaboration with scientists at Kyoto University in Japan, investigated the effects of increasing the levels of leptin, an appetite-controlling hormone. The mice had a severe deficiency of fat cells, which can cause a form of diabetes known as lipoatrophic diabetes. Patients with lipoatrophic diabetes lack body fat, have severe insulin resistance and elevated blood lipid levels, and eat excessively. One explanation for this is that leptin is normally produced by fat cells. When glucose metabolism changes in response to a meal, fat cells release more leptin, which then travels to the appetite control center in the brain and tells it to "stop eating!" Plasma leptin concentrations are markedly reduced both in patients with lipoatrophic diabetes and in rodent models of the disease. Earlier research had indicated that leptin, in addition to regulating appetite, can also act as an anti-diabetic hormone by increasing both glucose metabolism and insulin sensitivity. By genetically engineering mice to produce extra leptin in their livers, or by administering laboratory-made leptin to the animals, the scientists were able to improve the diabetic symptoms of the mice. Based on these findings, leptin may be a potential long-term therapeutic agent for the treatment of lipoatrophic diabetes.

These advances highlight the enormous contributions of mouse genetic models to our understanding of diseases such as diabetes. Discovering or defining the role of different signals in type 2 diabetes through such animal research is pivotal to understanding disease development and progression in humans. Through the creative use of mouse genetic models, researchers will continue to make discoveries about the signaling pathways involved in type 2 diabetes, enabling them to better target future research endeavors to yield improved therapies for this devastating disease.

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Stress Hormone May Determine Fat Deposition that Promotes Diabetes

Obesity is the consequence of greater energy intake as food than energy output as metabolism and exercise; the excess energy is stored as fat. However, the best predictor of obesity-associated diseases such as diabetes is not total body fat, but the amount of visceral fat—fat built up from deep within the abdominal area. One therapeutic approach to the complications of obesity could be to control where fat is deposited in the body. Recent research studies have indicated that the stress hormone cortisol may play a key role in determining where fat is deposited. Normally present in low amounts, active cortisol can be regenerated inside cells from an inactive precursor molecule by an enzyme, 11ß hydroxysteroid dehydrogenase type 1 (11ß HSED-1). In obese humans, one site of abnormally high activity of this enzyme is in fat cells. NIDDK-supported researchers recently investigated the significance of this enzyme activity in fat cells using a mouse model. They found that mice genetically engineered to make more 11ß HSED-1 in just their fat cells ate more than normal mice. Moreover, all of their fat cells, but especially their visceral fat cells, became bigger than fat cells in normal mice, resulting in visceral obesity. As the mice grew older, they also developed metabolic complications similar to those observed in obese persons, including insulin resistance and hyperlipidemia (increased lipids in the blood). There is evidence that one drug already in use to treat type 2 diabetes reduces visceral fat by repressing the activity of 11ß HSED-1 in fat cells. Strategies that repress activity of this enzyme, and hence reduce cortisol production, may therefore become an effective treatment for visceral obesity and its complications.

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Preventing Type 2 Diabetes and Its Complications

A person with diabetes and no known cardiovascular problems has the same risk of having a heart attack as a person who does not have diabetes but who has

already had a heart attack. What can individuals do to prevent the development of type 2 diabetes, its strong association with cardiovascular disease, and its many other devastating complications? Recent advances emerging from clinical trials are providing useful answers.

Impressive Clinical Trial Results Tranform Hope into Reality: Results from a major NIDDK clinical trial are providing important knowledge about diabetes prevention strategies. The Diabetes Prevention Program (DPP) demonstrated that individuals with impaired glucose tolerance and at risk of developing type 2 diabetes can prevent disease onset and improve their blood glucose levels through modest improvements in diet and exercise (see "Story of Discovery: The Ominous Link Between Obesity and Type 2 Diabetes"). Approximately 20 million Americans suffer from impaired glucose tolerance (IGT)—a condition in which blood glucose levels are higher than normal but not yet at diabetic levels. Left untreated, IGT often progresses to type 2 diabetes, and it is also associated with an increased risk of cardiovascular disease. Of the over 3,200 participants in the DPP, 45 percent were from minority populations who suffer disproportionately from type 2 diabetes—African Americans, Hispanic Americans, Asian Americans and Pacific Islanders, and American Indians. All participants were overweight, with impaired glucose tolerance, and were randomly assigned to one of the following groups: intensive lifestyle changes, treatment with the medication metformin, or a placebo control.

Lifestyle Interventions Have Enormous Benefits: The goal for the intensive lifestyle intervention group was to reduce weight by 7 percent through a low-fat diet and exercising for at least 150 minutes per week; the other two groups were also given information on diet and exercise, but there was no intensive lifestyle intervention. The study showed that the patients in the intensive lifestyle intervention group reduced their risk of developing type 2 diabetes by 58 percent. The lifestyle intervention was effective for both men and women and in all of the racial/ethnic groups. Lifestyle intervention also worked well in people over age 60, reducing the development of diabetes by 71 percent in this group. Participants randomized to treatment with metformin also reduced their risk of developing type 2 diabetes, but by 31 percent. Metformin was most effective in younger and heavier study participants.

Translating Message of Diabetes Prevention Program: This landmark study clearly demonstrated that, with instruction and encouragement, patients at high-risk for type 2 diabetes could be successful in improving their diet and activity levels and that these relatively modest changes had a major impact in reducing the onset of diabetes. Because of the strikingly positive results of the DPP, the NIDDK ended the study earlier than planned in order to disseminate its important prevention message as rapidly as possible to the public and to health practitioners. To this end, the NIDDK is expanding the National Diabetes Education Program (see sidebar: "Getting the Message Out: The National Diabetes Education Program"), which it supports in collaboration with the Centers for Disease Control and Prevention and 200 participating organizations in the private sector. The NIDDK is also supporting a cost-effectiveness study to determine the most efficient ways to achieve the translation of the DPP prevention message to the public and to health practitioners.

Post-DPP Study: Long-term follow-up of the DPP cohort will be undertaken to see how long the interventions will be effective. In addition, the researchers will further analyze the data to determine whether the interventions reduced cardiovascular disease and atherosclerosis, major causes of death in people with type 2 diabetes. The DPP cohort is the largest population of individuals with impaired glucose tolerance ever to be studied. The DPP was co-sponsored by the National Institute of Child Health and Human Development, the National Institute on Aging, the National Center for Minority Health and Health Disparities, the National Center for Research Resources, the NIH's Office of Research on Women's Health, and the Office of Behavioral and Social Science Research. The Centers for Disease Control and Prevention, the American Diabetes Association, and industry provided additional support.

Cardiovascular Complications: Heart disease is two-to-four times more common in diabetics than in non-diabetic adults. Women with diabetes are particularly at risk for heart disease. A recent analysis of data from the Nurses' Health Study revealed a dramatically increased death rate from heart disease associated with type 2 diabetes in women. As noted previously, diabetes confers nearly the same risk of death from heart disease as a previous heart attack. In other studies, researchers found that exercise can markedly reduce this risk. The American Diabetes

Association, the National Diabetes Education Program, and the National Cholesterol Education Program now recommend aggressive management of cardiovascular risk factors in diabetic patients to control cholesterol and high blood pressure and to address such other risks as smoking and obesity. These new epidemiological findings provide important insights into the prevention of cardiovascular complications of type 2 diabetes.

In a large study of risk factors for type 2 diabetes in women, researchers found that a healthy diet and lifestyle dramatically reduce the risk of type 2 diabetes. The researchers assessed weight, dietary, and lifestyle factors in nearly 85,000 women from the Nurses' Health Study. From these data, the scientists determined how combinations of risk factors were associated with a diagnosis of diabetes during the study, which lasted for 16 years. Excess body fat was the single most important risk factor for type 2 diabetes. Lifestyle factors were also associated with diabetes, including lack of exercise, poor diet, and smoking. While limited alcohol consumption correlated with decreased risk, the investigators cautioned against alcohol overuse. A healthy body weight, diet, and lifestyle also reduced the risk in women with a family history of diabetes. Encouragingly, even in overweight and obese women, a reduced risk of diabetes was achieved with exercise, a healthy diet, and abstinence from smoking.

These data complement the critically important prevention message from the recently-completed Diabetes Prevention Program (DPP) clinical trial. Both studies underscore the benefits that can be achieved from a healthy diet and exercise in preventing or reducing the risk of type 2 diabetes.

Diabetes in Pregnancy: Babies born to mothers with diabetes have an increased risk of becoming diabetic and obese themselves. However, it is not clear whether this is solely due to the genes inherited by the children, or whether the diabetic condition of the mother also plays a role. In a new NIDDK-supported study, researchers looked at families in which one child was born before and another after their mother's diagnosis with diabetes. The children born after their mothers had developed diabetes were more likely to be diabetic and obese themselves. Thus, the diabetic condition of the mother during pregnancy appears to affect a child's risk of diabetes and obesity. Since type 2 diabetes is increasingly occurring in younger women, the results of this study are particularly important, suggesting that prevention of diabetes in women of child-bearing age improves not only their health, but also the health of their offspring.

In addition to NIDDK's research efforts focused on women with type 2 diabetes, the NIDDK is also pursuing prevention and treatment of type 2 diabetes in children and adolescents. Recent epidemiologic data reveal an increasing number of cases of type 2 diabetes in the pediatric population, especially among adolescents and in certain minority populations. To address this alarming finding, the NIDDK is currently seeking research partners for the development of two separate clinical trials for the prevention and treatment of type 2 diabetes in children.

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Improved Long-Term Survival for Patients with Type 1 Diabetes

Type 1 diabetes affects nearly every organ system in the body causing serious complications and significantly reducing life expectancy. Indeed, before the introduction of insulin as a treatment in the 1920s, the onset of type 1 diabetes—usually in childhood—meant almost certain death. A later study of Americans who were diagnosed with type 1 diabetes between 1950 and 1981 found that these patients had mortality rates that were up to seven times higher than the general population. Since that time, great strides have been made in the medical care of diabetes such as the introduction of self-monitoring of blood glucose, the measurement of hemoglobin A1c, and better blood pressure management. However, little information has been available on trends in mortality from diabetes for patients diagnosed in more recent years.

Recently, researchers have examined the mortality rate within the Allegheny County (Pennsylvania) Registry of patients who were diagnosed with type 1 diabetes before their 18th birthdays between 1965 and 1979. Patients in this registry have been living with type 1 diabetes for an average of more than 25 years. By dividing the patients into three groups based on when they were diagnosed—those diagnosed between 1965 and 1969, between 1970 and 1974, and between 1975 and 1979—the researchers found clear evidence that survival rates for these type 1 diabetes patients had improved over time. For example, death rates between 10 and 20 years after diagnosis declined from 8.4 percent in the earliest group of patients to 3.5 percent in the latest. Importantly, both male and female patients showed equal improvements in survival. African Americans and Caucasians also both demonstrated improved outcomes, though mortality rates remained significantly higher in African American patients.

The encouraging results from this NIDDK-supported study suggest that research has led to improvements in medical care for type 1 diabetes, which have had a measurable impact on the survival of patients diagnosed in recent years. Continued follow-up of these type 1 diabetes patients will be necessary to document whether survival trends continue to improve over time. It will also identify reversible causes of mortality related to type 1 diabetes, suggest strategies to further increase survival, and help to find and address the causes of racial disparities in survival of patients with this disease.

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Beta Cells That Resist Destruction by the Immune System—Implications for Type 1 Diabetes

Great strides are being made in the search for techniques to help the insulin-producing pancreatic beta cells ward off attack and destruction by the body's immune system, and thus prevent the onset of type 1 diabetes in susceptible individuals. Beta cells appear to be particularly vulnerable to such autoimmune attack, as their ability to tolerate damage seems to be lower than that of other cell types in the body.

To gain insight into the role of the immune system in the onset of type 1 diabetes, NIDDK-supported researchers studied two genetically related strains of mice: the non-obese diabetic (NOD) mouse and the ALR/Lt mouse. Though these mouse strains share a common origin, NOD mice are highly susceptible to type 1 diabetes, while ALR/Lt mice are resistant to it. Thus, studies that compare these two mouse models may provide clues about the genetic signals that contribute to susceptibility to type 1 diabetes.

To better understand the reasons for ALR/Lt resistance to type 1 diabetes, the research team removed pancreatic islets, clusters of cells in the pancreas that include beta cells, from ALR/Lt mice. They then exposed these cells to chemicals that are known to promote inflammation and destroy the insulin-producing capacity of beta cells in NOD mice. Interestingly, ALR/Lt beta cells, unlike those from NOD mice, were able to maintain their ability to release insulin in the presence of these chemicals. Furthermore, ALR/Lt beta cells resisted destruction by the same type of T cells that kill NOD beta cells. To see if ALR/Lt beta cells could avoid destruction by the immune system in an animal, the researchers next exposed NOD and ALR/Lt mice to radiation that destroyed the animals' bone marrow, the part of the body that produces the cells of the immune system. Both sets of animals were then given replacement bone marrow from other NOD mice that had not been treated with radiation. Within 18 weeks after treatment with new bone marrow, all of the irradiated NOD mice had become diabetic. In contrast, no ALR/Lt mouse developed diabetes during this time, indicating that their beta cells were not destroyed by immune cells from the transplanted NOD bone marrow.

These experiments show that, far from being passive victims of the immune system, beta cells, such as those in ALR/Lt mice, can effectively fight off destruction by both chemical and biological agents. Importantly, when ALR/Lt mice are mated with NOD mice, their offspring are also resistant to type 1 diabetes. This observation strongly suggests that resistance of ALR/Lt mice to diabetes is a dominant genetic characteristic. Though the gene (or genes) that confers resistance is not yet known, identifying it could have important clinical implications for preventing type 1 diabetes in at-risk individuals.

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Type 1 Diabetes TrialNet

Knowledge of immune factors that contribute to the development of type 1 diabetes is now being used to identify individuals at risk for the disease and to design clinical trials aimed at preventing or delaying disease onset. A major new research program sponsored by the NIDDK, the Type 1 Diabetes TrialNet, has been initiated to develop and test strategies for prevention of type 1 diabetes. TrialNet will fund a consortium of clinical centers and core support facilities that will perform intervention studies with the purpose of preserving beta cell function from autoimmune attack. TrialNet will facilitate rapid, preliminary testing of emerging therapeutic strategies such as new ideas for immunoprevention. Those that prove most promising can then be moved quickly into larger-scale trials. TrialNet researchers will also complete the current oral insulin study of the Diabetes Prevention Trial for Type 1 Diabetes (DPT-1). The goal of the DPT-1 trial is to determine whether the use of oral insulin by non-diabetic relatives of individuals with type 1 diabetes can delay disease onset.

TrialNet will also be a valuable tool for identifying patients who may be able to help researchers find genes that predispose people to developing type 1 diabetes and diabetic complications. To further leverage the resources supported by TrialNet, biological samples and other data from participating patients may be placed in genetic repositories for use by many investigators. Thus, the NIDDK's TrialNet initiative represents a significant and promising investment in the ongoing search for methods to prevent and cure type 1 diabetes.

Prevention strategies tested in the TrialNet infrastructure will be particularly valuable to those at risk for type 1 diabetes. In this insidious disease, most symptoms do not begin until almost all of the pancreatic beta cells have been destroyed by immune system attack. Thus, by the time a person is diagnosed, damage to his or her beta cells is nearly complete. Finding ways to prevent or delay the onset of type 1 diabetes by interfering with this immune attack would be an enormous clinical achievement that would permit many people to avoid or reduce the severe health burden imposed by this disease and its complications.

Translating Diabetes Information into Intervention

In order to bring important research findings about diabetes prevention and control from "the bench to the bedside," effective strategies for translating these advances into clinical practice need to be developed continually. To this end, the NIDDK already supports a number of Diabetes Research and Training Centers (see sidebar on page 26), and has recently issued a research solicitation to promote diabetes prevention and control efforts. Through the support of both clinical and behavioral studies, this translational research program is expected to develop and test strategies for achieving objectives that have already been proven beneficial, such as control of glycemia and other risk factors for diabetic complications, and for enhancing behaviors that are expected to improve health outcomes for individuals with either type 1 or type 2 diabetes. This program will be especially supportive of interventions that focus on translating new advances into practice in under-served and minority populations.

The NIDDK has also established a program to develop diabetes-focused science education in American Indian tribal middle and high schools. The NIDDK plans to support faculty at Tribal Colleges and Universities and tribal community middle and high schools in the creation of an education program that will both increase awareness of diabetes and its risk factors and also highlight the role of science in the attainment of health and a healthy lifestyle. Through this initiative, the NIDDK hopes to increase the interest and competitiveness of American Indian students in pursuit of biomedical careers by exposing them to biomedical science through the prism of diabetes.

Inborn Errors of Metabolism

Just as insufficient amounts of an important signaling molecule, insulin, can lead to diabetes, deficiencies in metabolic enzymes can cause a number of devastating

disorders. Important research progress has been made in two inherited metabolic diseases in which inadequate levels of a needed enzyme lead to the storage of excessive amounts of normal biologic substances in cells and organs, resulting in toxicity. Through this work, the NIDDK is moving toward its long-standing goal of identifying the functional changes in the bodies of patients suffering from genetic metabolic diseases in order to develop and test possible treatments. By studying both the normal and abnormal proteins made from genes, scientists are determining their function in healthy individuals and trying to understand how faulty proteins cause disease.

The first inherited metabolic disease in which an advance has been made is Fabry disease. This is a rare disorder caused by insufficient amounts of an enzyme critical to the breakdown of fat in cells. Without sufficient enzyme, fat builds up and damages organs such as heart, kidneys, and brain. Eventually, untreated patients develop kidney disease, heart disease, and the potential for having a stroke. This year, investigators tested enzyme replacement therapy as a means for decreasing the levels of built-up fat. Two clinical trials demonstrated that patients treated with enzyme replacement therapy were able to reduce the amount of fat deposited in their heart, kidneys, and skin. The treatment also improved the patients' quality of life, and patients enrolled in the study opted to continue therapy after the study's conclusion.

Also within this past year, NIDDK-supported researchers identified two genes responsible for inheritance of a second, rare inherited metabolic disease known as Niemann-Pick Type C Disease. Cells of patients suffering from this disorder have faulty cholesterol transport, resulting in accumulation of cholesterol in the brain, liver, spleen, lungs, and bone marrow. The disease is characterized by an enlarged spleen and liver, poor muscle control, impaired eye movements, slurred speech, and dementia, and is always fatal, usually by age 15. The newly-identified genes each produce proteins critical to different aspects of fat transport. In people with Niemann-Pick disease, abnormal proteins are produced. Researchers found that adding normal protein to cultured cells taken from Niemann-Pick Type C patients restores normal cholesterol transport. Scientists are now challenged with developing a therapy to use this knowledge to treat victims of the disease.

Frustratingly, even when the causative genes and faulty protein functions are known, doctors are still unable to treat some genetic metabolic disorders effectively. In FY 2002, the NIDDK will sponsor a workshop entitled "Innovative Approaches to Therapy" to attempt to identify new treatment methods for inherited disorders considered "untreatable." Participants at this workshop will also discuss so-called genetic modifiers, or genes other than the faulty gene whose presence or absence can influence the severity of an inherited metabolic disease.

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Hormonal Regulation of Bone Loss

Hormones are major regulators of bone mass, and changes in hormone levels may lead to bone diseases such as osteoporosis. New developments in understanding hormonal mechanisms underlying the regulation of bone formation, on the one hand, and bone loss, on the other, make it possible to develop new therapeutic agents to properly regulate bone turnover and to potentially rebuild bone.

Bone is made up of about one third collagen and two-thirds mineral, the latter as crystals of calcium phosphate which harden the bone. About 99 percent of the body's calcium is in bone. But bone is actually a dynamic tissue, laced with the cells responsible for keeping a balance between bone formation and breakdown (resorption). When bone is formed or lost, blood calcium levels

change, signaling the parathyroid gland in the neck to restore calcium balance by secreting a hormone called parathyroid hormone, or PTH. Somewhat paradoxically, PTH can promote either the formation or loss of bone, depending upon the need; PTH also increases calcium absorption from the intestine (indirectly), and decreases the amount of calcium lost from the body in the urine. NIDDK-supported studies, highlighted in the "NIDDK Recent Advances and Emerging Opportunities (February 2001)," have elucidated how PTH is able to stimulate either bone formation or loss, suggesting the possibility for designing therapies to either suppress bone loss or increase bone formation. This was recently tested in two NIDDK-supported small-scale clinical trials, which demonstrated that using synthetic PTH for the treatment of osteoporosis can have a beneficial effect on bone mass.

In patients suffering from primary hyperparathyroidism, PTH levels are increased inappropriately, thus affecting bone loss and buildup. Increased PTH levels also result in excessive levels of blood calcium and can lead to kidney stones and other side effects. Primary hyperparathyroidism is diagnosed by a routine blood test that includes measurement of calcium levels, usually initiated because of the onset of symptoms ranging from weakness and fatigue, depression, or aches and pains, to loss of appetite, nausea, vomiting, constipation, confusion or impaired thinking and memory, and increased thirst and urination. When disease is confirmed by measurement of PTH levels, primary hyperparathyroidism is usually treated by surgically removing the parathyroid glands.

However, there are a large number of cases of so-called "asymptomatic primary hyperparathyroidism," in which individuals are still at risk for developing some of the negative side effects of primary hyperparathyroidism, including thinning of the bones, but often have not been diagnosed because of a lack of overt symptoms. Routine blood testing for calcium levels now identifies these patients, but what is the best treatment for mild disease? The long-term side effects of elevated PTH (and hence calcium) need to be evaluated against the long-term costs of surgery. In 1990, the NIDDK sponsored an NIH Consensus Development Conference, which deliberated how best to treat patients with asymptomatic hyperparathyroidism. The NIDDK has supported several important clinical studies addressing research questions identified at this meeting. In light of new information coming out of these studies, the NIDDK has scheduled a "Workshop on Asymptomatic Primary Hyperparathyroidism: A Perspective for the 21st Century," for April 2002, to generate an agenda for future research.

Highly Active Antiretroviral Therapy for HIV Infection—Benefits and Drawbacks

In recent years, the advent of highly active anti-retroviral therapy (HAART) has dramatically improved the survival of patients infected with the human immunodeficiency virus (HIV). HAART has been very effective in decreasing viral load, reversing the wasting syndrome, and prolonging survival in adults with HIV infection. Despite the clear benefits of the new anti-retroviral therapies, HAART has not been an unqualified success. This drug regimen, which often includes a drug known as a protease inhibitor, has been associated in many individuals with a variety of metabolic complications—including elevated levels of circulating fats and cholesterol in the blood, resistance to the actions of the hormone insulin, the development of osteoporosis and bone loss, and the abnormal distribution of body fat, or "lipodystrophy."

Lipodystrophy is a condition characterized by increased deposition of fat in the abdomen and trunk, and/or loss of fat in the face and extremities, and it appears to occur commonly in patients on HAART. These metabolic abnormalities represent major risk factors for the development of other serious diseases, such as diabetes and cardiovascular disease. Unfortunately, distress over these often disfiguring changes has caused some patients to stop taking anti-viral medications. The HAART therapy has also been increasingly associated with elevated levels of insulin in the blood and impaired glucose tolerance, ominous warning signs of possible impending diabetes.

Although HAART has allowed many HIV-positive people to prolong their lives, one of its unexpected and negative side effects is exposure to an elevated risk of diabetes and heart disease. The NIDDK is investigating the changes that HAART can cause in metabolism and is supporting studies of drug action. Current research efforts include studies designed to determine more precisely the health risks of these metabolic changes, their molecular basis, and to more fully understand the

negative role HAART can sometimes play in the treatment of HIV infection. These efforts may help to eliminate the negative side effects of HAART, which is a valuable therapy for many people with HIV infection, and, as another NIDDK study showed, can be quite beneficial to children who are HIV positive.

Increased Risk of Cardiovascular Disease in HIV-Positive Patients with Fat Redistribution Associated with Antiretroviral Therapy: In normal individuals who are not infected with HIV, elevated serum fat and cholesterol levels in the presence of insulin resistance or diabetes confer risk for the development of atherosclerotic heart disease. Because of this correlation, HAART-associated side effects are a serious potential public health concern. However, the cardiovascular risk in HIV-infected patients with this metabolic syndrome is unknown. One way to assess a person's risk of developing serious cardiovascular disease, including heart attacks and strokes, is to measure the levels of certain enzymes that circulate in the blood. Elevated levels of two particular proteins, plasminogen activator inhibitor-1 (PAI-1) and tissue-type plasminogen activator (tPA), are known to indicate elevated risk of future cardiovascular disease. NIDDK-supported scientists examined these proteins in a group of people who were HIV-positive and undergoing HAART in order to determine their relative risk of developing cardiovascular disease.

Scientists studied 86 HAART-treated HIV-positive patients who had experienced recent changes in body fat distribution. Thirty-three of these patients were found to have impaired glucose tolerance or elevated insulin levels in their blood, two risk factors for the development of type 2 diabetes. These patients also had elevated levels of PAI-1 and tPA, suggesting that they were at elevated risk for cardiovascular disease. A subset of these individuals was then enrolled in a study designed to determine whether the drug metformin, which is currently used in the treatment of type 2 diabetes, could be beneficial to HIV-infected patients with metabolic complications of HAART. After three months, patients who received metformin demonstrated significant reductions in the levels of PAI-1 and tPA as well as a return of their circulating insulin to a lower level. This result suggests that metformin may lower the cardiovascular disease risk in HIV-infected patients who develop metabolic complications of HAART.

Effect of the Protease Inhibitor Indinavir on Import of Glucose into Muscle: How might the metabolic effects of HAART be mediated? Scientists are studying this question in two ways. The body largely regulates circulating levels of glucose through the controlled uptake and release of the sugar in skeletal muscle and liver tissue. As described in preceding sections, the hormone insulin signals tissues to take up glucose from the blood after a meal to either use it or store it; later, when blood glucose levels have fallen, other hormones signal these tissues to release glucose into the blood. If HAART were somehow upsetting this balance, it would be a clue as to why some HIV-positive individuals on HAART develop metabolic complications, resistance to insulin, and, in some cases, type 2 diabetes.

In order to determine the impact, if any, of HAART on the import of glucose into skeletal muscle, NIDDK-supported scientists studied whole muscles isolated from rats. By incubating the muscles in a solution containing radioactive glucose, the researchers were able to monitor the rate at which the sugar was imported into the muscle by measuring the radioactivity incorporated into the tissues. The addition of insulin dramatically increased the amount of glucose imported into the muscle, a result in agreement with what happens in the body. When the muscles were incubated in a solution containing the protease inhibitor indinavir, a common component of many HAART regimens, the import of glucose following the addition of insulin decreased by 40 to 58 percent. Did the drug somehow interfere with the ability of insulin to signal the cell? When the scientists examined, at a molecular level, how the protease inhibitor diminished glucose uptake by the muscle, they found that insulin signaling was normal. However, when the cell attempts to import the sugar, it cannot; therefore, indinavir inhibits the transport of the glucose molecules across the outer cellular membranes but does not interfere with the ability of insulin to signal the cell.

Further evidence for a role of the protease inhibitor indinavir in glucose metabolism comes from a study of ten HIV-negative men who were given the drug for four weeks to determine its effect on glucose metabolism in healthy individuals. Indinavir therapy resulted in significant increases in fasting blood glucose levels, higher insulin levels, higher insulin-to-glucose ratios, and increased insulin resistance.

The cause of the HAART-associated metabolic syndrome is unknown. These two studies indicate that,

even in healthy individuals, the protease inhibitor indinavir can significantly impair glucose metabolism, suggesting that protease inhibitors may contribute to the metabolic changes seen in patients receiving HAART. This seems to be a result—at least in part—of impaired import of glucose in skeletal muscle. These studies offer important insights into the understanding of the physiological causes of HAART-associated metabolic complications and may lead to the refinement of HIV therapeutic approaches.

Protease Inhibitors Impair Fat Cell Development: Fat cells develop from an immature precursor cell that, under certain conditions, differentiates into a mature fat cell known as an "adipocyte." Using the appropriate signals, it is possible to coax pre-adipocytes to mature into adipocytes in culture, and such controlled differentiation is a powerful technique for studying the steps in the cellular differentiation process. Scientists have used this approach to study how protease inhibitors might influence the development of fat cells. Researchers found that, in the presence of the protease inhibitor nelfinavir, immature pre-adipocytes failed to differentiate. Moreover, when the drug was added to a culture of already-mature fat cells, they died. The results clearly demonstrate that, while nelfinavir is not toxic to pre-fat cells, it seems to diminish the number of mature fat cells through two mechanisms: by inhibiting the differentiation of pre-adipotyces and by promoting the death of mature adipocytes. Understanding the molecular basis of this and other metabolic changes associated with HAART may lead to the development of safer, more effective anti-HIV therapies that do not have the unwanted side effects on adipocytes that may play a role in the development of lipodystrophy.

Benefits of Antiretroviral Therapy in HIV-positive Children: Although HAART is not without negative side effects, this therapy has been responsible for some remarkable successes. Both survival time and quality of life have improved dramatically in HIV-positive children with the introduction of HAART. The growth patterns in HIV-positive children prior to the introduction of HAART indicate that they have similar birth weights compared with non-infected children but that they fall behind in both weight and height within the first months of life. Scientists have therefore been interested in determining whether HAART, and particularly the protease inhibitor component of HAART, could have a positive impact on these and other growth parameters in HIV-infected children.

Researchers monitored the growth of a group of HIV-positive children who were treated with drug therapy that included at least one HIV-1 protease inhibitor. After two and one-half years, treatment with protease inhibitors resulted in significant increases in weight, weight-for-height, and arm muscle circumference compared to status prior to therapy. A smaller effect was seen on height alone. Protease inhibitor therapy also reduced levels of HIV in the children's blood by nearly 80 percent. The use of protease inhibitors in the treatment of children with HIV infection therefore has a beneficial effect, resulting in improvement of several growth parameters. Whether the metabolic complications sometimes seen in adult HIV-positive patients receiving HAART with protease inhibitors might also appear in children will be monitored closely and will also be a topic of future studies.

NIDDK and NIH Research Efforts: Despite the clear benefits of the new anti-retroviral therapies, the metabolic abnormalities induced by the HAART regimen represent major risk factors for the development of other serious diseases, such as diabetes and cardiovascular disease, as well as bone fractures. Initial attention was focused on the protease inhibitors as the possible cause of these metabolic complications; however, the protease inhibitors are frequently used in combination with several other medications, making it difficult to pinpoint the "offending" agent. In addition, metabolic complications have emerged in patients who are not being treated with protease inhibitors.

Several large epidemiologic studies are currently ongoing with an eye towards producing a better description of the metabolic changes associated with HAART and understanding whether particular drugs, or classes of drugs, are the causative agents of these changes. In addition, a large research effort is aimed at understanding the molecular mechanisms by which anti-retroviral drugs might lead to these metabolic abnormalities. A long-term goal of this research is the development of new, highly active anti-HIV drugs that lack these adverse metabolic consequences.

In the meantime, it is essential to develop strategies to improve lipid levels and insulin sensitivity, to restore normal body fat distribution, and to minimize bone loss in patients treated with HAART therapy, in order to enhance patient compliance and to decrease the risk for

future disease. To foster research in this area, the NIDDK and the National Heart, Lung and Blood Institute (NHLBI) have announced a research program that seeks to develop and test strategies for treating the metabolic complications associated with anti-retroviral drug therapy in patients with HIV infection. The expectation is that clinical studies initiated under this program will both test the effectiveness of agents currently approved for the treatment of dyslipidemia, insulin resistance or diabetes, and osteoporosis, and also develop and test novel treatment approaches to the HAART-associated metabolic changes.

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Getting the Message Out: The National Diabetes Education Program (NDEP)

As the outcomes of so many clinical studies converge to underscore the fact that, in many cases, the onset and progression of type 2 diabetes can be prevented or slowed through early interventions, there is a pressing need to quickly disseminate the ensuing health recommendations to the general public. The National Diabetes Education Program (NDEP) is a collaborative initiative of the NIDDK and the Centers for Disease Control and Prevention that uses over 200 public and private partnerships to promote, through education, early diagnosis and improved treatment and outcomes for individuals with diabetes. A key feature of the program's partnership is the participation of individuals who represent communities of African Americans, Hispanics/Latinos, Native Americans/Alaska Natives, and Asian and Pacific Islanders, communities disproportionately affected by diabetes.

The NDEP is conducting a series of diabetes awareness campaigns using the theme, "Control Your Diabetes for Life." This theme is built on the landmark clinical trials that showed the importance of blood glucose control in preventing diabetic complications. By reinforcing this theme, the NDEP encourages patients with diabetes to manage the disease closely in order to live healthier lives. The campaigns target both general audiences and populations disproportionately affected by diabetes. Television, radio and print public service announcements, educational materials, and information kits for the media and communities, are helpful products of the NDEP. The program is currently developing campaigns to encourage health care providers to work with their patients to improve glucose control, and to identify, diagnose and treat children with type 2 diabetes.

The NDEP is also joining forces with the U.S. Department of Health and Human Services and the American Diabetes Association to inform the public that good diabetes management is more than just lowering blood glucose. Control of blood pressure and cholesterol is crucial to help prevent heart disease and stroke—the leading killer of people with diabetes. This new public awareness campaign comes in response to recent studies that show a dramatic link between diabetes and heart disease. Research now shows that people with diabetes can live longer and healthier lives with relatively small decreases in blood glucose, blood pressure and cholesterol. To communicate the importance of comprehensive care in simple language, the "ABCs of Diabetes" have been developed. The "A" stands for the hemoglobin A1c test, which measures average blood glucose over the previous three months. "B" is for blood pressure, and "C" is for cholesterol. This approach was developed because the vast majority of people with diabetes are not aware that they are at very high risk of cardiovascular disease and that this risk can be greatly reduced with appropriate treatment.

The NDEP is also the primary mechanism for translating the impressive results of the NIDDK's recently completed major clinical trial, the Diabetes Prevention Program (DPP), to the public and to health care practitioners (see accompanying text: "Preventing Type 2 Diabetes and Its Complications)." This multi-center trial showed that even a relatively modest exercise and weight-loss program could significantly prevent or delay the onset of type 2 diabetes in those at risk for this disease. Importantly, these findings applied across all ages and ethnic/racial groups studied (individuals from minority groups who are disproportionately affected by type 2 diabetes represented approximately 45 percent of the DPP study population). Based on these scientific results, the NDEP is now being expanded so that this critically important prevention message can be broadly translated.

Diabetes Research and Training Centers—Making a Difference in Diabetes Care

Grace Hill Neighborhood Health Centers, Inc. uses a model to evaluate its diabetes care that sounds like it is straight out of corporate America: Plan-Do-Study-Act. But its clients definitely are not corporate. The six clinics Grace Hill runs serve mainly poor African Americans in St. Louis, MO. "Eighty-six percent of our patients are uninsured," says Veronica Richardson, Grace Hill's director of chronic care management. "People we work with are sometimes just a paycheck away from being homeless," and some live in households where one income stretches to cover three generations.

Grace Hill is part of a 70-center network that gets help from the University of Chicago's Diabetes Research and Training Center (DRTC). "We work with them on quality improvement issues," says Dr. Marshall Chin, a researcher at the Chicago DRTC.

Like the five other NIDDK-funded DRTCs, the Chicago group does basic and clinical research and looks for ways to close the gap between what researchers know is good care and how patients are actually treated. Their Prevention and Control Divisions (formerly known as Demonstration and Education Divisions) "translate" clinical discoveries into clinical practice through education programs, professional training, and community outreach; they study the barriers that block ideal diabetes care, and they help providers assess their programs.

Most members of the network Chin works with are "safety net" providers supported by the Bureau of Primary Health Care, a federal agency whose mission is to improve the health of underserved populations. These providers work in resource-constrained settings, so delivering good diabetes care is challenging, says Chin. "If we can treat successfully in these settings, then we can probably change care in other settings."

"At Grace Hill, there's excellent leadership and a committed staff willing to try many interventions," adds Chin. "Because the centers are small and non-bureaucratic, they have the attitude and values that allow them to change quickly."

He says that the Plan-Do-Study-Act (PDSA) model is a way to try new interventions and to move on quickly, if they don't work. "The model provides a framework, then the clinics can get creative."

Grace Hill uses the model when it evaluates patient materials and management changes. They use several small cycles of testing before deciding to print large quantities of patient materials. For instance, they take steps as basic as checking the readability and cultural sensitivity of patient information with a few people. "We don't want thousands of copies of something to end up in the basement because they couldn't be used," says Richardson.

One of Grace Hill's more successful changes, the "group cluster clinic," was developed with PDSA. The cluster visit is a sort of one-stop shopping: patients come in and see all relevant health care providers at once. "It's an idea we got from another group that worked with all insured folks, but we weren't sure it would work for us," says Richardson. But they wanted to do something about the high no-show rate—it was more than 50 percent—that occurred when patients were scheduled for many separate visits.

The first attempts failed. The first diabetes cluster clinic was opened a few days before Christmas on the coldest day of the year. The staff learned not to schedule near a major holiday. Initially, they offered the clinic to groups that included homeless and non-homeless people. But the tensions between the two groups and their different needs ended that idea.

They also tried to do the cluster clinic for their homeless clients at the beginning of the month. But when that did not work well, "we moved it to the middle of the month," says Richardson.

The way the group cluster visit works now, the patients with diabetes come in first thing in the morning, go to the lab, get a healthy breakfast at the clinic, and then have a group education and support session. The nutritionist may talk, and people discuss what has worked in their own management of the disease.

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Meanwhile, explains Richardson, the staff looks at the records and decides which patients have to see which provider. The patients don't see every provider during every visit, but there's a general doctor, an eye doctor, a foot doctor, a social worker, and a health educator, says Richardson.

Grace Hill's patient registry is sophisticated enough to track when patients need to come in and for what tests, and it lets the providers know when someone has skipped a particular exam. From the registry, the staff can create lists for the health care coaches, neighborhood people hired by Grace Hill who meet one-on-one with clients to coax them into improving their care.

Doing It All

Translation work since 1977 has shown the diabetes community just how broad the care issues are, says Dr. Edwin Fisher, a psychologist at Washington University's DRTC in St. Louis. "Twenty-five years ago, it was assumed that good doctors, good nurses, and good curricula could solve the problem of diabetes. Now, we know that it involves the community, the public health people, psychologists, et cetera."

"We've done a great job at developing materials, but we're still not good at changing behaviors," adds Chin. "We have to do it all."

Doing it all means educating patient and practitioners, involving the greater community (see story, page 30), and even changing how the medical system manages patients. Over the years, the DRTCs have done numerous studies that tackle issues in each of these areas.

Helping the Patient

The DRTCs have developed patient education programs that address issues ranging from cultural relevance to motivation. For instance, the University of Chicago developed the "Pathways Lifestyle Modification Program for African American Women," a successful lay educator program designed for inner city African American churches, and the University of Michigan created "Living With Diabetes: Challenges in the African American Community."

Dr. Roland G. Hiss, chief of the Prevention and Control Division of the University of Michigan DRTC, thinks that a patient empowerment approach developed at Michigan "is the most important thing we've done in the nonbiomedical field." The approach is used in diabetes care in many places in the United States, and it is attracting interest in other countries, including Mexico, the United Kingdom, Germany, and Japan.

The underlying concept in patient empowerment is goal clarification, explains Dr. Robert Anderson, a Michigan DRTC researcher. The idea is to help patients find the first small step they want to take in their care and to explore their feelings about what will happen if they don't make changes.

Until a patient internalizes that 'I want to live a healthier life,' 'I want to be there for my grandchildren,' or some similar message, "then nothing happens. Motivation is a personal thing, and it can't be superimposed from the outside," says Anderson.

"We have to help patients discover their motivations," adds Anderson. He and his Michigan colleague Martha Funnell turned the approach into a book called *The Art of Empowerment*, which is published by the American Diabetes Association (ADA). They also have taken strategies from the book and tailored them for urban African Americans.

As they developed the empowerment principles, Anderson says the researchers wanted to make sure patients understood the clinical implications and consequences of their health choices and found ways to mesh their diabetes care and their lives. "We also help them discover whether their decisions are supporting their goals."

On the patient education side, there has been a greater recognition that people do what makes sense for their lives, says Fisher, so successful patient education has to emphasize flexibility and choice.

Fisher says years of behavioral study show that people who are more advantaged are better able to change their behaviors, probably because they have fewer barriers to care. Researchers know behavioral changes are more likely to occur in a person if they hear about its necessity from several sources. Also, the longer programs to support behavior change are maintained,

the longer those changes will persist, he adds. "Chronic behavior, just like chronic disease, needs chronic care."

Another determinant of long-term behavior change is encouragement and support. "But, as much as we know support is tremendously important in health, we don't know very much about what is supportive in what circumstances," says Fisher.

The Diabetes Control and Complications Trial, a 10-year, multi-center clinical trial that ended in 1993, had shed some light on motivation. DRTC researchers at Washington University found that patients who participated in the intensive arm of the study reported that they had received more non-directive support than directive support. (The researchers describe non-directive support as cooperating without "taking over" and accepting people's feelings and choices, and directive support as taking control and telling people what to choose or feel.) That patients reported more non-directive support from staff was "a bit of a surprise since the highly detailed and technical demands of the DCCT Intensive Treatment might have made it reasonable for staff to provide a lot of directive support," says Fisher, who adds that earlier studies show that non-directive support is associated with better metabolic control and quality of life. The DRTCs at the University of Chicago, Albert Einstein College of Medicine, Indiana University, and Washington University have begun studying the two forms of support to evaluate how they may help patients adhere to protocols in future studies.

One support that has greatly helped patients is the telephone. Dr. Elizabeth Walker, director of the Prevention and Control Division of the DRTC at Albert Einstein College of Medicine in the Bronx, reported a doubling of the screening rate for retinopathy among African Americans in a study using a telephone intervention. During the conversations between interviewer and patient, the interviewers would help patients find and overcome their personal barriers to screening. Perceived lack of time and acute health care problems that took precedence over preventative care were among the reported barriers. The researchers are now evaluating the cost effectiveness of the phone intervention in English and Spanish.

Dr. Judith Wylie-Rosett of Einstein's Prevention and Control Division has developed and evaluated strategies for providing weight control services in community settings. Among the strategies are a workbook, now published by ADA, and a computer program. Both help people prioritize weight loss strategies that might be helpful to them. Wylie-Rosett hopes to take further advantage of technology by using the Internet. One idea is to use cyber cafes in schools and other sites to address the rise in youth obesity and diabetes.

Supporting patients requires understanding their specific needs, according to Dr. Loretta Heuer, diabetes coordinator of Migrant Health Services, Inc, in Moorhead, MN. She and her colleagues serve Hispanic workers who move to rural Minnesota and North Dakota to work the farms every summer. When their labor is done, they go home to Texas. Of the 6,000 patients served, approximately 400 have diabetes, and the most common medical reason their patients visit is for diabetes care, says Heuer.

To stay on top of patient care, the nurse-managed clinics in Minnesota remain in contact with clinics that serve the migrant workers back home in Texas. They have also trained members of the community to be lay educators. "When they go back to Texas, the lay educators restart support groups," says Heuer.

The group collaborates with the DRTC at the University of Chicago. "We provide them with data on how to make patient visits more effective," says Heuer.

The program for the migrants runs on federally funded vouchers that pay for medical care such as visits to physicians and dentists, laboratory tests, x-rays, and medications. The patients receive the voucher and referral from Migrant Health Services as needed, says Heuer. "The vouchers make it possible to provide good care."

Because it is a voucher program, the nurses and nurse practitioners can spend enough time with patients. It's not unusual for them to take an hour or more during a visit, which gives the patients enough time to start building trust, says Heuer. "It's a culture where it would be offensive to have a short visit."

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The building of respectful relationships can only improve care, according to Heuer, who adds that she and her colleagues are seeing drops in hemoglobin A1c (A1c) levels in their patient population. "It makes you feel like you are not running upstream."

Teaching Providers

Several DRTC projects have worked to improve the ways providers deliver diabetes care. Materials have been developed for dieticians, doctors, diabetes educators, and other members of the diabetes care team.

For instance, at Einstein, Wylie-Rosett has collaborated to develop the WAVE interview, which helps time-pressured physicians make faster nutrition assessments of patients. WAVE stands for weight, activity, variety (varying time of day for carbohydrates), and excess (looking at excessive behaviors around carbohydrate consumption). "We've used WAVE to train medical students to integrate a five-minute screening nutrition evaluation into the care of women with gestational diabetes," says Wylie-Rosett. "Physicians who address nutrition are more likely to refer patients for needed medical nutrition therapy."

One focus of the Indiana University DRTC researchers involves training future doctors and established practitioners. In one project, they worked with second year medical students who had never touched a patient. The idea was to teach them to make better decisions about diabetes care. The students would receive the patients' charts and decide what to do. Then they would watch videos with real patients and experienced doctors doing the examination. Finally, one of the doctors shown in the video debriefed the students. "We put them into a clinical simulation and made them get smarter," says Dr. David Marrero at Indiana University's DRTC. "They came up with good treatment ideas, and their performance was comparable to the fellows in diabetes."

Efforts have been made to educate whole offices and established physicians using in-service training, chart audits, and other methods with mixed results. To bring about change at this level, "we need to look at reimbursement," says Marrero.

"It's very tough to change doctors who have been out many years," adds Hiss, who likewise thinks changing how diabetes care is reimbursed may bring about improvements in care. He thinks translation of diabetes findings also would be improved if the U.S. healthcare system could be reorganized to provide a "chronic disease model" of care for diabetes. Under such a model, care is planned—everything a diabetic patient might need in terms of tests, education, specialist visits is regularly scheduled—and there is a strong emphasis on prevention either of the disease or of its complications. Most medical care is now delivered under an "acute care model," where treatment is often in response to crises.

"The most effective diabetes care programs address patient, practitioner, and [the health care] system," says Dr. Charles Clark, director of the DRTC at Regenstrief Health Center in Indianapolis, IN, who recently worked with a Las Vegas-based managed care organization to improve its diabetes care.

With intensive management over a year, the managed care organization was able to make improvements in several areas. For instance, the number of patients in the high-risk category (A1c greater than or equal to 8.0 percent) decreased by 58.3 percent; approximately 97.4 percent of the people in this risk category had a change in their treatment regimen during the study. Among the other successes: more people had their blood pressure checked regularly, and there was a decline in hypertension. There was also a decline in the percentage of patients in the highest risk for coronary heart disease (LDL greater than 130 mg/dl) from 25.4 percent at baseline to 20.2 percent.

"We had patient, physician, and system changes happening," says Clark, who details the study in the June 2001 issue of *Diabetes Care*. The doctors increased their compliance with the Health Plan Employer Data and Information Set 1999 Diabetes Quality Improvement Project measures, which define how often patients should have dilated eye exams, foot exams, urinary microalbumin measurements, lipid profiles, and A1c tests. Patient risk for cardiovascular disease, nephropathy, and other conditions was

assessed, and patients and doctors received the assessment reports. Patients attended programs that educated them about their risk status and learned what actions were necessary to prevent problems, and the health team coordinator studied patient records monthly using a computer application to track timing of interventions.

There was a good deal of satisfaction with the Las Vegas program, says Clark. Even one change, like making a printout of risk, made a difference because "the doctors weren't wasting time convincing patients there was a problem—they could see there was one. Patients also felt that the physicians were paying attention." Clark hopes to try a large-scale version of this study in the future.

One of the significant questions is how could changes like those that occurred in the Las Vegas study be carried over to solo practitioners, says Clark. When doctors at a rural practice in Clinton County, IN, asked Clark and his colleagues for help, the practice managed improvement for about six months. After a year, most of the improvements had disappeared, possibly because there hadn't been a strong patient education component.

"If any one piece falls out, it doesn't work. We need activated patients, a practitioner with guidelines, and systems that educate patients and support practitioners," says Clark.

Working in the Community

Diabetes is more common in African Americans than whites, and "in our area, African Americans are more likely to have diabetes than African Americans elsewhere," says James Pichert, director of the Clinical Outcome and Behavioral Sciences (COBS) Core at Vanderbilt University's DRTC in Nashville, TN. "It may be because of cultural factors. It may be that the gene pool for diabetes is deeper here." Whatever the reason, Vanderbilt's DRTC researchers are especially interested in learning what it will take to eliminate the racial disparities in the prevalence of diabetes in their region and elsewhere.

There is no textbook on how to change a community's attitudes toward diabetes, says Pichert. But there are ways that researchers can be more successful when they work with communities.

An educational psychologist by training, Pichert was hired to do research on how to promote diabetes knowledge. To work, any educational strategies that researchers develop have to be appropriate to the community and practical enough to be used.

To come up with good strategies, Pichert thinks researchers need to do their homework and read everything they can about the community in which they hope to work; they need to make friends who are well connected in the community, and they need to keep showing up. Judge Mattielyn Williams, an administrative law judge for the state of Tennessee and an advocate for better diabetes care in African American communities, is an important connection for the researchers. "She took it upon herself to help us at Vanderbilt get plugged in," says Pichert.

Williams accompanied Pichert and colleagues to meetings with ministers; when someone needed a health speaker, she'd often team up with Pichert; when he gave talks, she'd debrief him and let him know when his message was off the mark. She kept sending Pichert back into the community, even when he felt as though he had embarrassed himself. "She told me, 'you have to go back. Only if you keep showing up, will people begin to trust you.'"

For meaningful health changes to occur in a community, there has to be a coalition that backs the idea; attention has to be given to the supports and barriers to the changes, and behavioral support systems have to be put in place to keep positive changes going, says David Schlundt. He directs the DRTC's COBS Core and Vanderbilt's Behavioral Health Disparities Core,

which is based at Nashville's Meharry Medical College, the training ground for many of the country's African American physicians and dentists.

The Vanderbilt researchers began building ties in the community in the early 1990s. By 1997, a coalition had formed with the aim of reducing health disparities. The Nashville Disparities Coalition now includes Matthew Walker Comprehensive Health Center, Meherry Medical College, Fisk University, Tennessee State University, the county hospital, local and state health departments, the Nashville Branch NAACP Health Community, ministers, concerned citizens, and the Vanderbilt DRTC. "It had its seeds in the American Diabetes Association African American Initiative and came to a head with NIH's emphasis on health disparities," says Pichert.

The coalition has made possible a series of research collaborations between Schlundt and Meharry colleagues on African American women's eating habits and a project funded with a Centers for Disease Control and Prevention REACH (Racial and Ethnic Approaches to Community Health) grant. The grant to the coalition has Matthew Walker Health Center as the lead agency; the DRTC has a subcontract with the center to provide evaluation of programs, says Schlundt. He says that Vanderbilt's social science expertise, built with DRTC funding, has been one of its primary contributions to the coalition. "We've also supported the coalition in community building and in obtaining resources."

The members of the coalition have worked especially hard to promote the community's readiness to change. Under the direction of Michelle Marrs, CEO of the Walker Health Center, and her colleague Linda McClellan, community-based REACH teams of health educators and outreach workers are making people aware of health disparities in their community by using data provided by Nashville's Metropolitan Health Department. Public service announcements, surveys in schools, cookbooks, referral services, and stories in community newspapers have raised awareness. A popular local disc jockey talks about his diabetes on the air, and the REACH teams make presentations at community events and work with a local managed care group to promote better screening and with local transportation providers to get people to services, says Pichert.

When Schlundt and the REACH teams looked at barriers to treating or preventing diabetes, they tackled problems in pieces. For instance, "we want to promote exercise, but focus group participants told us they don't go out because of problems like broken street lights or mean dogs on the street. We are beginning to work with the city to improve street conditions."

Smoking is bad enough for health, but it's deadly when combined with diabetes, says Pichert. To reduce smoking among juveniles, REACH team members are working to limit the practice of selling loose cigarettes. In some poor communities, shopkeepers sell cigarettes one at a time, making them affordable to children. "The goal is to let store owners know that it is a violation to sell to children. If local people would tell the police about it, we can encourage an officer to swing by and give warnings," says Pichert. The local police chief, who is African American and interested in community policing, supports the effort.

When it comes to creating behavioral support systems, the REACH teams have worked with local shops to offer "REACH sandwiches," healthy, lower fat choices. They've worked with the Interdenominational Ministers' Fellowship to identify pastors interested in health. The goal is to help pastors and their churches' health committees or nurses' guilds to sponsor activities like cooking and exercise classes. Because the churches have standing in the community, people are more likely to go to their programs.

Only the people in a community can tell researchers about issues—like the loose cigarette sales—that are specific to their neighborhoods, says Pichert. He says the coalition partners believe that the researchers' role is to help empower the community to identify those problems, set priorities, find potential solutions, and evaluate results, but "not tell our colleagues and neighbors what to do." Once the community decides what its

Diabetes Research and Training Centers

Working in the Community

health priorities are, researchers can help them understand the scientific basis for necessary changes and assess programs once they come into play. "We can then feed the data back to the community, so they can decide what needs to be done next," says Pichert.

On a personal note, Pichert sees work in this field as "an opportunity to make a generational difference." When the disease strikes older African American women many people feel the impact, says Pichert. "One of my favorite research assistants had to drop out of Vanderbilt and go home because his diabetic grandmother had a stroke and could no longer care for his grandfather."

"We'd like to see the grandmothers, who are the glue of the community, have far, far fewer diabetes-related strokes and amputations," says Pichert.

"It will be several years before we can even hope to see if what we are doing has an impact on major health outcomes like stroke," adds Schlundt. "We hope to see changes in behavior first, but even that will take a couple of years."

Because there is no one formula to change a community, the DRTCs continue to search for methods that will work at the local level.

The Ominous Link Between Obesity and Type 2 Diabetes

Americans are facing an epidemic of obesity and type 2 diabetes, according to epidemiologic studies. Type 2 diabetes, a devastating illness already afflicting 90 to 95 percent of the 16 million people who have diabetes,[1,2] can lead to serious complications including blindness, kidney failure, lower limb amputations, and heart disease. Although genetic factors may predispose a person to be overweight or develop diabetes, other factors must also be involved, because our genes could not possibly have changed quickly enough to account for the rapid increase in the prevalence of obesity and type 2 diabetes. Research indicates that the obesity problem essentially results from Americans' eating too much and exercising too little. But how is an increase in obesity related to an increase in diabetes? For a long time, scientists have known that obese or overweight people are far more likely to develop type 2 diabetes; in fact, 80 percent of patients with type 2 diabetes are overweight or obese. However, only recently have scientists begun to find the biological molecules that connect these two health problems.

Type 2 diabetes develops through a multi-stage process. First, the body becomes unable to use insulin effectively, a condition known as insulin resistance. Insulin is a protein made by cells in the pancreas called beta cells. Insulin normally helps the body maintain a healthy level of the sugar glucose in the blood by causing fat and muscle cells to store glucose and by reducing glucose production in liver cells. When insulin resistance develops, the beta cells try to compensate by making more insulin. For a while, this helps keep blood glucose levels relatively normal, but eventually the beta cells become exhausted and cannot produce enough insulin to overcome the insulin resistance. At this point, individuals develop a condition called impaired glucose tolerance, in which blood glucose levels are higher than normal but not as high as those in diabetes. Left untreated, however, this condition frequently progresses to full-blown type 2 diabetes. Unfortunately, people with insulin resistance and impaired glucose tolerance experience no outward symptoms and thus are unaware of this silent progression towards diabetes.

How is obesity connected to insulin resistance and diabetes? Clues are being found in unexpected places. Surprisingly, fat cells are not passive storehouses for fat, just keeping fat in case it is needed for energy. Instead, fat cells actively sense changes in energy availability and signal the brain and other tissues to regulate feeding and cellular processes. Scientists are learning that fat cells send out these signals in the form of special hormones, or signaling proteins, which the fat cells make and secrete. With the discoveries of novel signaling hormones, scientists are learning that the connection between fat and diabetes involves a complex balance of fat cell hormones.

Among the signaling proteins made by fat cells are leptin, resistin, and adiponectin (also known as Acrp30). After a meal, fat cells release leptin. This hormone signals the appetite-control center in the brain to stop eating. Scientists found that mice lacking the gene for leptin overeat and become obese. When given leptin, these mice lose weight—unfortunately, however, administering leptin to people does not effectively treat obesity. Thus, additional factors must also contribute to obesity.

Resistin, another fat cell signaling protein discovered recently, is so-named because too much of this hormone is thought to cause insulin resistance. When scientists gave mice a substance that inhibits resistin activity, their blood sugar level and insulin response improved. In fact, scientists initially discovered resistin as a result of some creative experiments to investigate how fat cells are affected by anti-diabetes drugs called TZDs, which are used to treat people. One of the results of adding a TZD drug to fat cells turns out to be decreased resistin production—and improved response to insulin. Thus, resistin itself might now be useful as a

target for the discovery of new anti-diabetes drugs.

Another protein produced by fat cells, adiponectin, appears to connect obesity and diabetes in a way opposite to that of resistin—while too much resistin apparently causes insulin resistance, too little adiponectin may also be problematic. Scientists working on mouse models of obesity and type 2 diabetes recently found that giving extra adiponectin protein (made in the laboratory) to the mice caused them to become less insulin-resistant, lowering their blood glucose levels to near normal. In other experiments, when scientists gave mice a TZD drug, the type of diabetes drug that lowers resistin levels, they found that the drug can also increase adiponectin levels. From other research in mice, it appears that adiponectin helps muscle cells burn more energy; it also reduces body weight. In people, studies suggest that overweight and diabetic patients do not produce enough adiponectin. Thus, adiponectin may also be a good target for new therapies. In light of these studies, the ominous link between obesity and diabetes may be a balance of the levels of several fat cell signaling proteins with different effects. As more is learned about fat-cell signaling proteins, new drug therapies can be developed for obesity and diabetes.

While basic scientists are learning about what causes obesity and type 2 diabetes at the molecular level, clinical researchers are developing other measures to combat these conditions. Results from an exciting new study give us a way to battle the epidemic of obesity and type 2 diabetes. A major clinical study demonstrated that patients at risk of developing type 2 diabetes can prevent disease onset and improve their blood sugar through modest improvements in diet and exercise. These results are particularly important to minorities, who made up 45 percent of the study participants and are at increased risk of developing diabetes.

This study, called the Diabetes Prevention Program (DPP), identified overweight individuals suffering from impaired glucose tolerance, a condition which, as discussed, increases the risk for type 2 diabetes. In the study, patients were assigned to one of three groups: intensive lifestyle intervention, medication, or placebo control. The latter two groups also received conventional information about diet and exercise. The intensive lifestyle intervention had a goal of reducing body weight and staying active with a minimum of 150 minutes of exercise a week. The lifestyle intervention worked the best; patients in this group reduced their risk of developing diabetes by 58 percent. Significantly, the intensive lifestyle intervention was highly effective for both genders and all ages and racial/ethnic groups in the study. Patients in the medication group, who received the diabetes drug metformin, were 31 percent less likely to develop diabetes than the control group, and they also lost weight. Metformin, while less effective overall in reducing the risk of diabetes, was effective in both genders and in all the racial and ethnic groups, which included African-Americans, Hispanic Americans, Asian Americans, and American Indians. In contrast to the lifestyle intervention, however, which was highly effective for all age and weight groups, metformin was not as effective among patients who were less overweight and was not effective in people over 60. This landmark study showed that with instruction and encouragement, patients at high risk for diabetes could be successful in improving their diet and activity—with these relatively modest changes having a major impact in reducing the onset of diabetes.

1. "National Institute of Diabetes and Digestive and Kidney Diseases." *Diabetes Statistics.* 19 December 2001 http://www.niddk.nih.gov/health/diabetes/pubs/dmstats/dmstats.htm#prev

2. "Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion." *Diabetes Public Health Resource: National Diabetes Fact Sheet.* 19 December 2001 http://www.cdc.gov/diabetes/pubs/facts98.htm#appendix

PATIENT PROFILE

Sigrun Schwendinger—Islet Transplantation Holds Great Hope for Type 1 Diabetes Patients

Sigrun Schwendinger lived with type 1 diabetes for 50 years. Diagnosed at age 7, she took insulin daily to keep her blood sugar levels within normal range—and to stay alive. Today, at age 57, Sigrun is insulin free thanks to a revolutionary new treatment originally developed by researchers at the University of Alberta in Edmonton, Canada, and now reproduced by a team at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and National Institutes of Health (NIH) Clinical Center. Commonly referred to as the "Edmonton protocol," insulin-producing beta cells found in clusters called "islets" are extracted from the pancreases of deceased donors and then transplanted into patients with type 1 diabetes, who then are treated with an experimental combination of immunosuppressive drugs. So far, only a few people worldwide have undergone this experimental yet promising new transplant technique. In fact, Sigrun, having undergone the procedure at the NIH Clinical Center in December 2000, is the first person in the United States to have been successfully transplanted using the new protocol. "I feel extremely fortunate that I qualified for this procedure, and that my prayers of years ago to be insulin free have been answered," she says.

Islet transplantation using the new protocol, however, is still very much in its infancy. No one knows, for example, what the outcome will be five, ten, or twenty years from now for people who undergo the protocol today. But scientists both at the University of Alberta in Edmonton, Canada, and the NIDDK are hopeful that the new islet-cell research may eventually lead to treatment not only for type 1 diabetes patients, but for some type 2 patients, as well.

Sigrun Schwendinger, 57, underwent pancreatic islet transplantation in Winter 2000-2001. As a result of this procedure, Sigrun says: "I'm very hopeful for not only my future, but for the future of the millions of others who suffer with type 1 or type 2 diabetes."

FACT:
An estimated one million Americans suffer from type 1 diabetes; an additional 15 million have type 2.

Living with Diabetes

Type 1 diabetes results when the body's immune system destroys the pancreatic insulin-secreting beta cells that control blood sugar (glucose) levels. As a result, people with type 1 diabetes fight a constant battle to keep their blood glucose levels from going too low or too high. People with type 1 diabetes must

"manage" the disease by taking daily injections of insulin—sometimes as often as four or five times a day, depending on their glucose levels—and by controlling their dietary intake.

FACT:

Even those who "manage" their diabetes well are at high risk for heart disease, stroke, and nerve damage. Diabetes is also the leading cause of kidney failure, blindness (in adults), and non-traumatic amputations, and shortens average life expectancy by up to 15 years.

Unlike most with the disease, Sigrun, an admissions assistant at a private school, says diabetes did not hamper her lifestyle for many years. Until recently, she required only one shot of insulin a day. Even as a teenager, Sigrun says "I never had a craving for sweets, and when I did have something like pudding, I'd only take one spoonful, and no more." Also, she was fortunate in that she never suffered any early complications as a result of her diabetes. She married, gave birth to and raised three healthy, non-diabetic sons, and, for the most part, led a normal life—until around the age of 50.

"Everything was going well," Sigrun says. "After menopause, however, my blood sugar periodically would spike to 300 in a matter of hours (a non-diabetic normal range is between 80 and 120 after a meal)." Every couple of weeks she experienced rapid heart beat, excessive perspiration, and felt confused due to extremely low blood sugar levels. Although Sigrun never had serious kidney ailments, by her mid 40s she began manifesting symptoms of nerve and eye diseases associated with diabetes, and had cataracts removed from both of her eyes. After years of successfully living with diabetes, "I suddenly became more frightened of my situation," she says, "and its terrible side effects."

Edmonton Protocol

Islet-cell transplantation is not new. Over the past 25 years or so, more than 300 patients have undergone such transplants in medical centers around the world. But only a few were successful, and very few if any proved effective long term (beyond one year). Most scientists believe that the poor long-term success rate has been due to the body's rejection of the transplanted cells.

The scientists in Edmonton, Alberta, Canada, developed a clinical protocol that uses a novel, steroid-free combination of three drugs. The drug combination appears to prevent rejection as well as halt autoimmune destruction of the islets. In this technique, islets are isolated from the pancreas of organ donors. Following isolation, the islets are injected into the portal vein, which supplies blood to the liver. The islets then migrate to the liver, where they flourish and produce exactly the amount of insulin required to maintain almost perfect blood sugar control. A high percentage of patients who have been transplanted using this new protocol have remained insulin free. As a result, the approach taken in the Edmonton protocol is now being tested in a larger number of patients.

Research to Increase Supply of Islets

One of the limiting characteristics of the Edmonton protocol is that it usually requires two or more pancreases to yield sufficient islets for each patient. Should the protocol become more commonplace, the demand on an already short supply of donor organs will inevitably increase dramatically. Scientists at the NIDDK and elsewhere already are trying to induce islet cells to reproduce in laboratory cultures. They also are attempting to determine whether or not animal stem cells can be programmed to grow into islets.

Becoming Insulin-Free

After going through an extremely rigorous screening process that included filling out a lengthy questionnaire and meeting with several physicians, Sigrun underwent a battery of tests, including EKGs, stress tests, and insulin tests. She was eventually placed among the NIDDK's list of 60 candidates for the procedure. "I was

advised of all the risks involved," says Sigrun, including blood clots, and side effects from a depleted immune system. Sigrun emphasized the fact that she was told repeatedly by her NIDDK research physician that she could leave the protocol at any time. But with the support of her husband and family, Sigrun decided to go through with the procedure, and as a result, played a part in the history of this new transplant technique.

Because the procedure is so new, researchers don't know what complications might arise over time. "Therefore, the biggest risk is the unknown," says David Harlan, MD, Chief of the Transplantation and Autoimmunity Branch of NIDDK, who attended to Sigrun during her transplant. He adds that, while Sigrun has benefited from her new-found and—hoped for—long-term independence from insulin, she also has contributed toward the development of a treatment that may one day legitimately be called a cure for type 1 diabetes. "Not only has she helped win a victory for humanity," he says. "Sigrun also afforded me the privilege I have long sought. That is, she was the first patient I was able to look in the eye and say, 'Congratulations, you no longer, at least for today, have diabetes.'"

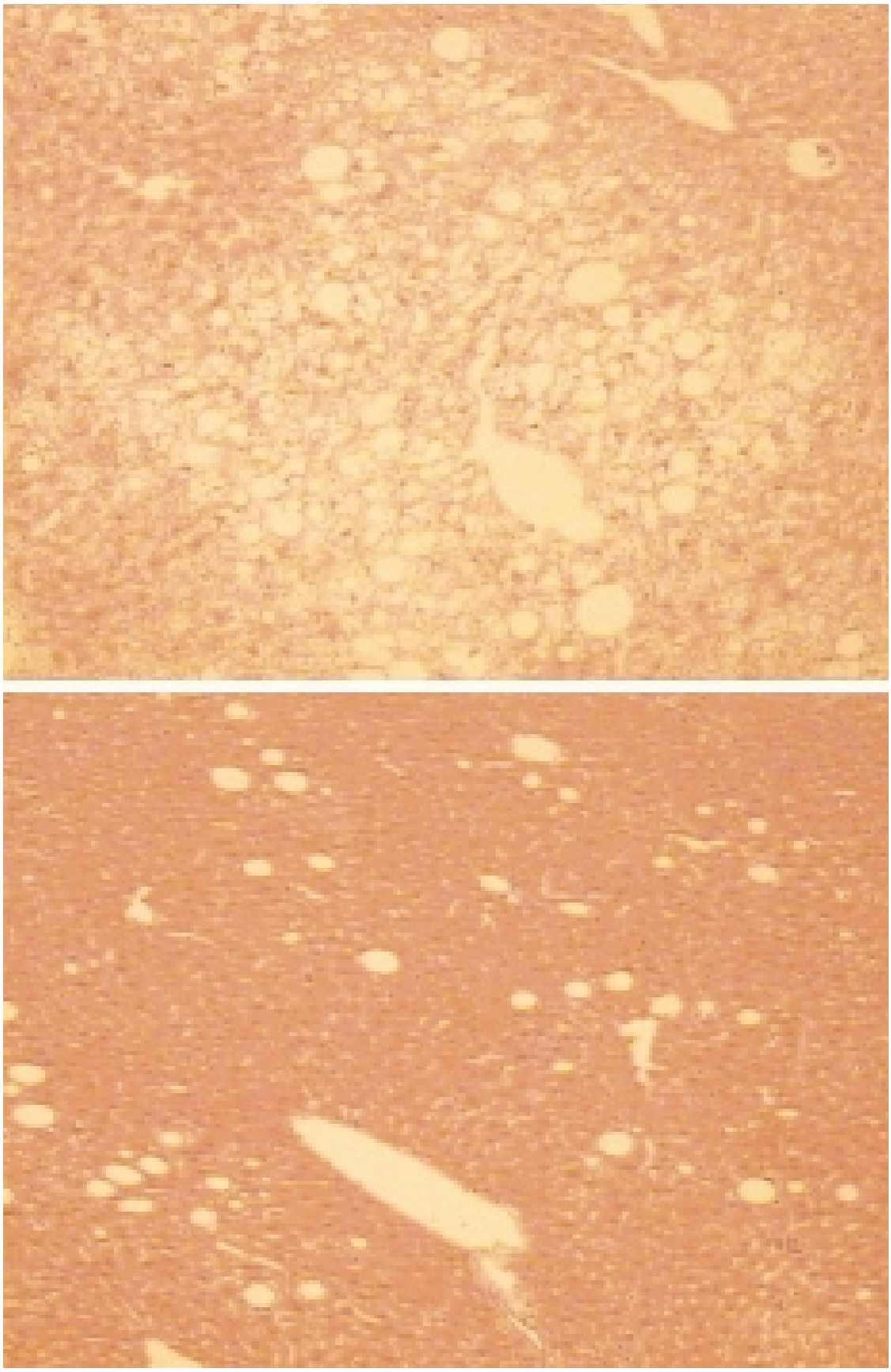
As this document goes to press, it has been eleven months since Sigrun underwent the two-stage islet transplant procedure—and she remains insulin free. NIDDK physicians monitor her condition on a regular basis, and she continues to take immunosuppressant drugs. She says the dosage of these drugs is being gradually reduced as time goes by.

Sigrun, who describes herself as an optimistic, confident, cheerful person, as well as a risk taker, admits that she had given up all hope years ago of being cured of diabetes. "As a result of this procedure, and the follow-up I am receiving at NIDDK, I'm very hopeful for not only my future, but for the future of the millions of others who suffer with type 1 or type 2 diabetes."

Undergoing Islet Transplantation

A single donor pancreas provides about 250,000 to 500,000 islets. Each recipient patient, however, needs about 800,000 cells before he or she is insulin free. As a result, the patient normally needs two infusions of cells, from two donors.

In Sigrun's case, after being called in for her first infusion, she was sent home because researchers were unable to isolate enough islets in the lab. A week later, another donor organ of her blood type was received and she was able to undergo the first infusion. It took a month and a half before a second suitable organ was found to complete her islet transplant. Between the first and second infusions her dosage of insulin was reduced by half. A day after the second infusion, she was insulin free.



This photograph shows the fat content (white) of the liver from mice deficient for the hormone leptin (top) and those therapeutically treated with the hormone (bottom). Leptin is an important regulator of appetite in the brain, and also alters fat metabolism in the liver and other tissues. Photo: Dr. Ken Ebihara, Kyoto University Graduate School of Medicine. Reprinted with permission from *Diabetes*, Vol. 50, 2001: 1440-1448.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of hospitalization, surgery, and disability in the U.S. They include disorders of the gastrointestinal tract, liver, gallbladder, and pancreas. Nutrition research is important to understanding, treating and preventing many diseases such as type 2 diabetes, obesity, chronic renal disease, heart disease and cancer.

Obesity: A National Health Problem

Obesity is the most common and fastest growing health problem in the U.S. Individuals who are overweight or obese are at heightened risk for developing a number of diseases, including type 2 diabetes, heart disease, stroke, and some forms of cancer. The U.S. Surgeon General recently issued a report, "The Surgeon General's Call to Action to Prevent and Decrease Overweight and Obesity," and publicly identified obesity as the second most common cause of preventable deaths in the U.S. Hence, questions arise regarding the effects of diet and lifestyle changes on quality of life and disease outcomes. Several ways of measuring overweight or obesity exist, including Body Mass Index, or BMI (see the accompanying sidebar on different measures, "Who Should Lose Weight?"). BMI is a ratio derived from a person's weight and height; people with a BMI of 25-30 are considered overweight, while those with a BMI higher than 30 are classified as obese. Based on BMI, more than half of adults in the U.S. are overweight, and nearly one quarter are obese.

Prevention Message of Type 2 Diabetes Trial, the Diabetes Prevention Program (DPP): Long-term studies have already emphasized the importance of diet and lifestyle in the primary prevention of coronary disease. Results that have recently emerged from the Diabetes Prevention Program (DPP), a major clinical trial supported by the NIDDK, vividly illustrate the importance of weight control in preventing another disease, type 2 diabetes. Participants in the DPP were overweight Americans with impaired glucose tolerance; 45 percent of the participants were from minority groups who are disproportionately affected by diabetes. In studying whether type 2 diabetes could be prevented in these individuals at high-risk for the disease, investigators compared the effects of: (1) intensive lifestyle intervention; (2) treatment with the drug metformin; and (3) placebo treatment; patients in the latter two groups also received conventional information about diet and exercise. Participants randomly assigned to intensive lifestyle intervention increased their physical activity by exercising for at least 150 minutes a week, usually with walking or other moderate exercise. They also lost five-to-seven percent of their body weight (an average of 15 pounds). As a result, this group reduced their risk of developing type 2 diabetes by 58 percent. These results demonstrate that even modest weight loss through diet and exercise can prevent a disease that is the main cause of kidney failure, limb amputations, and new onset blindness in adults, and a major cause of heart disease and stroke.

The Biology of Obesity

Obesity is a consequence of greater energy intake in the form of food calories than energy expenditure through metabolic processes and physical activity. The excess energy is most efficiently stored by the body as droplets of fat within specialized cells—adipocytes, or "fat cells"—that develop and grow in different parts of the body. Understanding the biology of fat cells will ultimately lead to improved approaches to managing and preventing obesity. As evidenced by several recent advances, investigators are learning a great deal about how the body regulates energy balance, how normal cellular factors might be manipulated to decrease body fat, and how both the environment and an individual's

genetic makeup can affect whether an individual becomes obese in the first place.

Tipping the (Molecular) Scales: How does the body normally know when it has enough energy stored to maintain a healthy weight? Like a car, the body takes in fuel (food), stores the fuel energy (mostly as fat), and burns it (through metabolism and physical activity). Also like a car, the body has gauges and indicators to assess its energy needs. Different tissues in the body release hormones and other molecules during exercise, fat metabolism, feeding, and stress. The brain constantly receives these signals, many of which indicate either an energy surplus or an energy deficit. The brain then responds with signals—also hormones and other molecules—that promote energy balance, usually by modifying behaviors such as feeding and physical activity. Many of these signals are received and generated in a region of the brain called the hypothalamus, considered the brain's "appetite control center." Much recent research has been devoted to identifying the molecules the body uses to gauge and respond to changing energy levels. For example, mice engineered to lack insulin receptors only in their brain eat more and exhibit elevated levels of body fat in contrast to normal mice. This finding indicates that insulin, which is produced by the beta cells of the pancreas, acts on the brain and plays a role in influencing food intake in addition to its better-characterized roles in glucose metabolism. Other hormones, such as the "fat hormone" leptin, target the hypothalamus to regulate body weight by either stimulating or suppressing appetite.

Imbalances in Hormonal Signals: If the fuel gauge fails to register a full tank in a car, the driver can keep adding gas unnecessarily; if the tank could expand, it would take over the car. Not surprisingly, researchers are finding that similar imbalances between the brain and body signals regulating feeding behaviors, energy storage, and energy expenditure can result in the "energy surplus" that leads to obesity. For example, melanin concentrating hormone (MCH) is a peptide hormone produced in the hypothalamus. Levels of MCH go up in mice when they are fasting, because levels of leptin go down; when MCH increases, mice eat more. NIDDK-supported researchers have found that mice genetically engineered to produce just twice as much MCH as normal mice in response to fasting signals eat more than normal mice, become obese, and develop insulin resistance. These results suggest that imbalances in MCH production may contribute to obesity and associated metabolic complications.

Scientists at the NIDDK have also recently found out more about how MCH production is regulated, through research on cellular receptors. Receptor molecules inside and on the surface of a cell act like radio antennae, picking up molecular signals in the cell's environment for translation into useful information. For example, insulin bound to the insulin receptor tells a cell that there is a lot of free glucose in the body. Cells use such information to decide what "molecular activities" they should engage in, such as altering their metabolism or releasing their own signals. Mice that have been genetically engineered to lack the M3 receptor, a specific type of cellular receptor for the signaling molecule acetylcholine, eat less than normal mice and are lean, despite having a normal metabolic rate. Importantly, these lean mice have abnormally low levels of leptin, but, unlike normal mice, their levels of MCH are not increased in response to this fasting signal. As it turns out, the M3 receptor is normally found on the very same cells in the hypothalamus that produce MCH. This finding suggests that acetylcholine may play a pivotal role in stimulating the production of MCH in response to low leptin levels. An exciting new role is thus emerging for acetylcholine, a molecule usually associated with memory, muscle stimulation, and gland secretion. These and future findings about the molecules involved in energy balance could lead to specific interventions for eating disorders that contribute to obesity.

Enhancing Calorie-Burning: Finding ways to enhance the body's burning of calories, called thermogenesis, is another therapeutic approach to obesity. Under normal conditions, the breakdown of fat is "coupled" to the production of chemical energy for use by the cells of the body. Because of this, people who want to lose weight are advised to modify their diets, in order to decrease the amount of food energy they consume, and to exercise, in order to increase the amount of energy they expend. When this happens, the body uses stored fat as an energy source. However, there may be other ways to achieve the dissipation of stored fat. In some fat cells, the presence of "uncoupling proteins" severs the link between fat metabolism and chemical energy production, and the energy that usually drives a series of chemical reactions is instead dissipated as heat. NIDDK-supported researchers have found that mice genetically engineered to overproduce an uncoupling protein in their skeletal muscle have

elevated rates of metabolism in both resting and active states, and are leaner than their normal counterparts. Complementing these findings, human genetics researchers in Europe have found a correlation between a genetic variation that increases uncoupling protein production in fat cells and a decreased risk of obesity.

The "uncoupling story" isn't so simple, however: NIDDK-supported researchers recently studied mice that were genetically engineered to under-produce or entirely lack an uncoupling protein that is normally found in a number of tissues, including the insulin-producing pancreatic beta cells. These mice did not gain weight, indicating that removal of the uncoupling protein does not contribute to obesity. Instead, the mice had lower resting blood glucose levels and higher levels of insulin, and they also secreted more insulin than normal mice in response to a "glucose challenge." Beta cells secrete insulin in response to increased chemical energy production, usually initiated by increases in blood glucose. In type 2 diabetes, insulin secretion eventually declines as these cells become insensitive to blood glucose levels. Thus, reducing, rather than increasing, the amount or the activity of the uncoupling protein in beta cells may be important in treating type 2 diabetes, a disease strongly associated with obesity. With better understanding of uncoupling proteins, it may be possible to search for drugs that carefully and specifically manipulate levels of these proteins in target tissues so as to shunt excess calories into heat, rather than fat production, while also maintaining or increasing insulin production by beta cells, thereby preventing or controlling both obesity and diabetes.

Role of Genetics and the Environment: Obesity is not a single disorder, but a diverse group of conditions with multiple causes. As some scientists identify the major regulators of energy balance in the body, others are looking for genetic and environmental factors, such as diet and stress, that influence or disrupt the normal pathways affecting fat accumulation.

Whereas some environmental factors, such as a consistently high-fat diet combined with low physical activity, will cause just about anyone to become overweight, some people have pre-existing genetic variations that will exacerbate the effect of environmental factors. For instance, a distinct correlation exists between increasing percentage of Native Hawaiian ancestry and development of obesity and diabetes. In the general population, over 200 genes, genetic "markers," and chromosomal regions have been reported that are possibly linked with BMI, body fat, and other obesity traits and complications. NIDDK-supported researchers recently studied the relationship between heredity and obesity in several hundred Caucasian families, scanning the entire genome for chromosomal locations that influence the development of visceral obesity, overall obesity, and insulin resistance. They identified two regions, or "loci," one on chromosome 3 and one on chromosome 17. One is strongly "linked" to several traits associated with obesity, including BMI and insulin resistance, and the other is strongly linked with levels of leptin. Such genetic linkage studies in large numbers of families can provide researchers with better maps to find the genes that have the most significant influence on obesity.

Identifying the environmental factors that promote obesity in the population is also extremely important in preventing the onset of disease, especially for individuals with a greater genetic susceptibility toward weight gain. Environmental factors may in fact be the single most significant cause of weight gain, as obesity has only become a significant problem in the post-industrialized world. Even more tellingly, the incidence of childhood obesity and type 2 diabetes has increased dramatically in the past decade.

The most obvious environmental factors affecting weight gain are those affecting diet and physical activity, such as access to high fat foods and an increasing number of sedentary occupations. Researchers are also examining other factors, especially ones affecting early development of obesity. For example, NIDDK-supported scientists recently reported that breast-feeding of infants reduces their risk of becoming obese in older childhood and adolescence. Such a finding could result in future recommendations for improved child health.

Obesity usually develops over a number of years, and is a reversible condition; however, its associated complications and diseases, such as diabetes and cancer, can cause permanent damage. Thus, as researchers identify the environmental factors that promote obesity, they are also developing strategies for early intervention. Because of the cultural and socio-economic influence on factors contributing to obesity, different approaches are also being tailored for specific populations and communities. The effectiveness of all of these approaches in preventing obesity and promoting health needs to be evaluated. In

collaboration with a number of other institutes at the NIH, the NIDDK is supporting an initiative, "Environmental Approaches to the Prevention of Obesity," a research solicitation that will establish studies of preventive approaches that target environmental factors contributing to inappropriate weight gain in children, adolescents, and adults.

NIDDK Efforts: The NIDDK maintains a strong program of research on and related to obesity, both as a serious risk factor for type 2 diabetes and its complications and as an independent health problem. The Institute established a National Task Force on the Prevention and Treatment of Obesity, which provides science-based guidance to aid research strategies and to generate public health messages. The NIDDK also supports Obesity/Nutrition Centers and Clinical Nutrition Units. A multicenter clinical trial has just begun that will examine the health effects of voluntary weight loss in obese diabetic patients, with particular emphasis on cardiovascular health. The trial is called "Look AHEAD," Action for Health in Diabetes. The NIDDK's public education efforts related to obesity include the Weight Control Information Network, and the National Diabetes Education Program. The latter is a cooperative initiative with the Centers for Disease Control and Prevention and approximately 200 public and private partnership organizations. Finally, the NIDDK supports all of these programs with a solid base of fundamental research on biologic processes such as nutrient metabolism and how it is influenced by genetic and environmental factors.

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Copper Transport

Copper is an essential nutrient for most living organisms. Catalyzing the movement of electrons within biological molecules, copper works with proteins as a cofactor to facilitate a variety of metabolic functions. These include photosynthesis and respiration, eradication of toxic elements, connective tissue formation, iron metabolism, and neurological function. Although copper is essential, it is also toxic. Only trace amounts of this metal are needed by the body and it is critical that proper levels of copper be maintained to prevent abnormalities and death.

The tragic effects of deficiencies or excesses of copper are demonstrated by two genetic diseases, Menkes disease and Wilson disease. Patients with Menkes are unable to secrete copper from any of their cells. Therefore, the cells lining the intestine, which can still absorb copper from digested food, cannot make it available to the rest of the body, resulting in copper deficiency. Children with Menkes disease suffer from growth retardation, severe neurological impairment, mental retardation, seizures, and hair and bone abnormalities, usually leading to death before age five. Wilson disease, in contrast, is the result of copper excess. Although patients with Wilson disease do not exhibit symptoms early in life, by age 40 most have suffered liver failure and severe brain deterioration associ-

ated with copper deposits in those organs. The genes responsible for Menkes and Wilson disease were recently identified. They code for two distinct but nearly identical enzymes, MNK (Menkes) and WND (Wilson), that are required for the proper export of copper from cells.

Insights from Yeast: To elucidate the mechanisms of copper transport by cells, scientists have historically used yeast, which are single-celled organisms, as a "model system." They are now using their findings in yeast to investigate how multi-cellular organisms maintain proper copper balance, or homeostasis, using animal models and state-of-the-art technology. Following the identification of the protein responsible for copper uptake in yeast, Ctr1p, investigators identified a genetically similar, or homologous, protein in plants, mice, and humans. These Ctr1p "homologs" can substitute for the yeast Ctr1p when they are introduced into yeast that have an inactivated *Ctr1* gene, indicating that they do act as copper transporters. To explore the role of the Ctr1 protein in multi-cellular organisms, NIDDK-supported researchers recently used genetic engineering to create mice in which either one or both copies of the murine *Ctr1* gene were "knocked out." Embryos without a functioning *Ctr1* gene died *in utero*, while those with one active copy of the gene seemed normal, but had approximately half the content of copper in their brains as normal mice. This evidence correlates well with the high expression of Ctr1 protein found in the mouse brain. Like the Ctr1 protein, the mouse MNK protein is highly expressed in the brain. The findings of this study suggest that both Ctr1 and MNK may play a crucial role in copper transport in the brain during development and adulthood.

Chaperone Proteins: New insights into copper transport inside cells have been gained through the recent identification of metallochaperones, a family of proteins that play a role in this transport process. The delivery of copper to intracellular targets in mammals appears to require the metallochaperone Atox1. To clarify the specific function of Atox1 in mammalian cells, and its role in copper stability, NIDDK-supported investigators used genetic engineering to create mouse strains in which the *Atox1* gene was knocked out, so that no Atox1 protein was made. Most of these mice could not survive long after birth and those that did exhibited growth failure, flacid skin, and seizures due to copper deficiency. The research team found that copper was delivered normally to the placenta of both mutant embryos and normal control mice, but significantly less copper was then transported into the *Atox1* mutant embryos. Fetal cells line the blood vessels of the placenta and are responsible for transferring copper from the placenta to the embryo. In the case of the *Atox1* mutants, these cells can take up copper but are unable to transfer it to the embryo. Thus, the mouse embryos suffer from copper deficiency during their development, with devastating results. Furthermore, these effects were compounded when the mouse mother also did not make any Atox1 protein. These findings are consistent with previous research demonstrating that mammalian Atox1 protein interacts with both the Menkes and Wilson copper transporting proteins, and they suggest that one important role for Atox1 is to deliver intracellular copper for export.

As demonstrated by these studies, copper homeostasis is essential from embryogenesis through adulthood in most organisms. Each step toward the elucidation of the role of copper and the mechanisms by which homeostasis is attained brings us closer to therapies and cures for devastating diseases involving copper imbalance.

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Hereditary Pancreatitis

Hereditary pancreatitis (HP), a rare genetic form of pancreatitis, was recognized as a distinct disease in 1952. Since its recognition, more than 200 families with HP have been identified. Studies of these HP families led investigators to determine that mutations in the gene coding for the protein, cationic trypsinogen, were responsible for their disease. Three different mutations in the cationic trypsinogen gene that clearly predispose patients to acute and chronic pancreatitis have been identified.

Trypsinogen, which is made by cells in the pancreas, is an inactive form of the digestive enzyme trypsin.

Normally, the pancreas secretes trypsinogen into the small intestine, where it becomes "activated" to trypsin. Trypsin then activates other pancreatic "pre-enzymes" secreted into the small intestine. The cationic trypsinogen gene mutations identified in HP patients interfere with this process by causing premature activation of trypsinogen while it is still in the pancreas. The mutations apparently either make trypsinogen unable to "auto-digest" itself, which it normally does when it is inappropriately activated in the pancreas, or simply enhance its premature activation. The presence of active trypsin in the pancreas results in the activation of the other digestive enzymes, causing destruction of pancreatic cells and subsequent pancreatitis. Inflammation of the pancreas causes severe abdominal pain, nausea, and elevated pancreatic enzymes. Individuals with hereditary pancreatitis have a lifetime risk of pancreatic cancer ranging from 40 to 75 percent.

HP mutations are "dominant," meaning that only one copy of the cationic trypsinogen gene must be changed for the disease to be expressed. However, only 80 percent of individuals who inherit a gene mutation develop the disease. This correlation led researchers to hypothesize that other modifier genes or environmental factors may contribute to the onset of pancreatitis. To explore this possibility, NIDDK-supported researchers used "twin studies." Twin studies offer a powerful tool for teasing out the role of genetic factors from the effects of environment in the development of disease, because identical twins have the same genetic information at the DNA sequence level. In such studies, identical twins are compared to sibling pairs in the same environment and to paired, unrelated individuals in a different environment. In this study, the overall rate of disease development among individuals in 14 separate twin pairs was nearly 80 percent, as is seen in large family studies. However, three pairs of twins were "discordant," meaning that one member of each pair, although carrying a mutation, did not develop disease. Differences in the age of onset in the other groups compared with the twins suggested that environmental factors or modifying genes may be important in disease expression, but these factors alone do not explain why 20 percent of individuals with mutations never develop the disease at all. The results of this study suggest that other, possibly non-heritable factors (such as unique events in an individual's experience that could act as "triggers"), may contribute to an individual's developing pancreatitis.

This research provides a vital analysis of HP patients and the contribution of genetics and the environment to their disease. However, other pieces of this puzzle are required to understand fully the mechanisms of hereditary pancreatitis. Solving the puzzle is expected to lead to new therapies that will minimize the symptoms or prevent the onset of this disease.

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Irritable Bowel Syndrome: Altered Neurological Activity

Irritable bowel syndrome (IBS) is a common disorder of the intestines that leads to pain, intestinal gas, bloating, and changes in bowel habits. People with IBS may have constipation or diarrhea and some people experience both. Other symptoms include the urge to move the bowels but an inability to do so. The cause of IBS is not known, and as yet there is no cure. IBS is classified as a "functional disorder" because there is no sign of disease when the colon is examined. Although it does not cause permanent harm to the intestines and does not lead to intestinal bleeding of the bowel or to a serious disease such as cancer, IBS patients suffer a great deal of discomfort and distress.

The underlying physiologic cause of IBS is unknown. Ordinary events such as eating and distention from gas or other material in the colon can cause the colon to overreact in a person with IBS. Individuals with IBS seem to have a colon that is more reactive and sensitive than usual, so it responds strongly to stimuli that would not bother most people. Researchers have found that the colon muscle of a person with IBS begins to spasm after only mild stimulation. Stress may also be a factor in the manifestation of disease symptoms.

Patients with IBS also seem to have an enhanced awareness of and sensitivity to normal gastrointestinal events, such as muscle contractions and the filling of the viscera following a meal. This has led some researchers to speculate that when the brains of persons with IBS receive information from the visceral nerves in the intestines, they may process the information differently than persons without IBS.

To examine the possible role of information processing

by the brain in causing IBS, researchers studied brain activity in affected patients. In the experiment, the scientists recruited twelve people with IBS, as well as twelve healthy volunteers, and inserted a catheter through the rectum and into the volunteers' colons. Each catheter contained two small balloons along its length that could be inflated to a precise pressure by the researchers. Inflation of balloons of this size is designed to produce mild discomfort, but no serious pain or tissue damage. After the catheters were inserted and the patients had a brief recovery period, the researchers initiated a Positron Emission Tomography (PET) scan of the patients' brains. This scan permitted researchers to see relative rates of metabolism—based on energy usage and blood flow—within specific regions of the brain. The researchers then initiated a three-part experimental phase, in which they told patients that the balloons would or would not be inflated, but did not consistently inflate the balloons in the manner stated. This experimental design allowed the researchers to see responses to no inflation (phase 1), an expected and delivered inflation (phase 2), and an expected but undelivered inflation (phase 3). During this procedure brain activity was monitored by PET scans.

When the scientists analyzed the data generated by the PET scans, they found significant similarities between the normal and IBS patients, but also noted important differences. Brain regions activated by actual and simulated balloon inflations were similar in both groups; however, differences in three important areas of the brain could be detected. First, patients with IBS exhibited enhanced activation of right prefrontal cortex in response to actual or expected balloon inflation, whereas in normal patients, both sides of the brain reacted to a similar extent. This region of the brain is thought to be very important for "higher" cognitive functions, including concentration and judgment. Second, within the anterior cingulate—an area deep within the brain thought to be involved in emotions such as sadness—an enhanced reaction was seen in IBS patients in a sub-region associated with the perception of pain and unpleasantness. Third, the IBS patients demonstrated an overall decreased activation of circuits in the brain believed to activate fear and defense responses.

All three of these observations indicate that IBS patients show altered brain responses to rectal stimuli, regardless of whether these stimuli are actually delivered or simply anticipated. This study provides solid evidence of altered brain activity in patients suffering with this syndrome that is of unknown origin. To help foster more research into the causes of IBS—and its possible treatments—the NIDDK is working with members of the IBS community to develop a conference on the topic of fecal and urinary incontinence that is relevant to many of the quality of life issues that have an impact on people with IBS.

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Hepatitis C

It is likely that infection with the hepatitis C virus (HCV) is the major cause of cirrhosis and end-stage liver disease in the U.S., responsible for 8,000 to 10,000 deaths per year and at least 30 percent of all liver transplants performed in adults in the U.S. Several studies have now shown that viral resistance to the current optimal therapy of alpha interferon and ribavirin in patients with chronic hepatitis C is two-to-threefold more common among African Americans than non-Hispanic Caucasians. The reasons for this difference are not clear, and unfortunately, studies of antiviral therapy have included too few African American patients to either measure the response rate to current therapies or analyze the factors responsible for the lack of effect of therapy. The NIDDK held a workshop on "Hepatitis C and African Americans" in December 1999, which confirmed the scant participation of African Americans in clinical studies.

New Clinical Trial To Address Resistance to Current Treatments: To address this issue, the NIDDK recently initiated a multi-center clinical trial that will study viral resistance to interferon alpha therapy in patients with chronic hepatitis C, specifically focusing upon African Americans. The Study of Viral Resistance to Antiviral Therapy of Chronic Hepatitis C (Virahep-C) is intended to administer first-time treatment to 200 Caucasians and 200 African-Americans with chronic hepatitis C who are infected with HCV genotype 1. Patients will undergo an extensive initial medical evaluation and then receive what is judged to be the current optimal therapy of chronic hepatitis C. During treatment, patients will be followed intensively for

virological, immunological, cytokine-signaling, and host genetic differences. It is anticipated that the study will help explain the diversity in clinical outcome of therapy for hepatitis C.

HALT-C Trial: Another important hepatitis C trial that has been initiated by the NIDDK is the study of "Hepatitis C Antiviral Long-Term Treatment to Prevent Cirrhosis (HALT-C)." This is a seven year study of therapy for hepatitis C focusing on patients with advanced disease (with severe fibrosis or cirrhosis) who have not responded to conventional therapy and for whom there are no other practical options available. This trial should help to determine whether progression of hepatitis C could be halted or modified in individuals who previously were virologic non-responders to treatment. Patients will initially receive a combination of long-acting (PEGylated) interferon and ribavirin. Those who continue as non-responders, presumed to be about 80 percent of the initial treatment group, will then be randomized to receive either PEGylated interferon alone or a placebo. Patients are being intensively studied for both beneficial and adverse effects. This trial is designed to enroll over 1,200 patients, with enrollment scheduled to be completed by December 2002. To maximize the knowledge that can be gained from this trial, the Institute has also sought the development of ancillary studies that will be co-funded with other NIH components that share a mutual research interest in hepatitis C. Using data collected before, during and after therapy, the ancillary studies will focus on such areas as the non-invasive assessment of liver fibrosis; how the hepatitis C virus replicates; risk factors for progression, including nutrition, obesity, smoking, and alcohol; and the role of genetic diversity in diagnosis and clinical management of hepatitis C.

Who Should Lose Weight?

Am I overweight or obese? At first, it seems like an easy question to answer. However, defining overweight and obesity proves more difficult than might be expected. At what point do the extra pounds cease to be an annoyance and become a serious threat to health? As Americans become heavier and heavier, the toll of obesity-related diseases such as diabetes and cardiovascular disease becomes greater. To appreciate the impact of excess weight on disease, one must realize that overweight and obesity are conditions that are defined by more than just total body weight as shown on a bathroom scale. Because of this, several methods to measure body mass and body fat have been developed.

Body Mass Index: Among health care professionals, perhaps the best known method for assessing body size is the body mass index, or BMI. BMI is a value derived from a person's height divided by his weight. Specifically, weight in kilograms is divided by height in meters, squared. Persons with a BMI of between 25 and 30 are considered to be overweight, while those with a BMI greater than 30 are classified as obese. For example, a person who is six feet tall and weighs 175 pounds has a BMI of 23.7, a value that is within normal range. If a person of the same height weighed 200 pounds, his BMI would rise to 27.1, indicating overweight. At 230 pounds, his BMI would be 31.2, indicating obesity. BMI represents a valuable and easy-to-calculate manner of determining whether a person is obese, and BMI may be used by both men and women to estimate their relative risk of developing disease.

$$\text{BMI} = \left[\frac{\textit{Weight(kg)}}{\textit{Height(m) x Height(m)}} \right]$$

Waist Circumference: Although BMI is a widely used and valuable tool, it is not perfect. Individuals whose weight is predominantly muscular, as well as pregnant women, may have elevated BMI values even though they are relatively healthy. Because of these and other limitations of BMI, scientists and physicians have looked for alternative ways to assess body fat in order to determine the likelihood of disease development. Studies have shown that people whose fat is primarily localized in their abdomens—with so-called "apple" shape—are at greater risk of developing complications, in particular cardiovascular disease, than individuals of the same weight whose fat is distributed in their hips and thighs—with so-called "pear" shape. These differences in the distribution of fat have led to another method for identifying individuals at risk—using a simple tape measure to determine waist circumference. In men, a waist circumference of 40 inches or greater places individuals at risk of developing a number of obesity-related diseases; in women, a waist circumference of greater than 35 inches is considered unhealthy. Importantly, many men store their fat in their abdominal region, in contrast to many women, whose fat is more likely to be deposited in the thighs and gluteal region. Although women tend to have more body fat than men, the fact that men are more likely to store it abdominally means that the fat in men may pose a greater health risk than that in women.

Comparative Measurements: The waist-to-hip ratio, a comparison of waist and hip circumferences, provides important information not only about the amount of fat a person carries but the proportion of abdominal fat and, by extension, relative risk of cardiovascular complications. People with a higher ratio are at increased risk of developing diseases associated with overweight. This measurement is informative because it provides a somewhat more refined measure of overall fat distribution. In general, men with a waist-to-hip ratio of greater than 1.0 and women with a ratio greater than 0.8 are considered to have an excess accumulation of fat in their abdomens. For example, a woman with a waist measurement of 30 inches and a hip measurement of 40 inches would have a waist-to-hip ratio of 0.75. In a recent study, women with a ratio greater than 0.76 had twice the risk of developing coronary disease than those whose ratio was 0.75 or lower.

Other Ways To Measure Body Fat: Another way to measure body fat is to look at subcutaneous fat—the fat

beneath the skin. This measurement is obtained using calipers, pincher-like devices that determine the thickness of the subcutaneous fat layer. The Standardized Skinfold measurement involves measuring the thickness of several defined folds of skin sampled at fixed points along the body. Based on a mathematical formula, the thickness of these folds is used to compute a person's approximate body fat.

Conclusions: Although methods of measuring overweight and obesity may vary, it is clear that excessive weight poses a serious risk to health. While the cut-off points in each measurement may seem arbitrary, they represent an effort to quantify an essentially imprecise variable. Each method for determining body fat has advantages and disadvantages, and no single value should be examined without considering the overall health of the individual. However defined, overweight and obesity contribute to the development of a number of debilitating diseases, including arthritis, heart disease, and diabetes. For example, the increasing prevalence of weight problems among young people is thought to be a driving force behind the alarming rise of type 2 diabetes in children. It is entirely possible that, if untreated, such individuals could face many years fending off in mid-life the serious complications of diabetes, including blindness, amputation, and kidney failure. It is therefore of vital importance that the problems of overweight and obesity be addressed aggressively by researchers, physicians, and patients.

Genetic Breakthroughs in the Study of Crohn's Disease

In a landmark finding, researchers announced the discovery of the first gene that confers susceptibility to Crohn's disease, a debilitating form of inflammatory bowel disease affecting an estimated 500,000 Americans. A targeted, interdisciplinary collaboration among scientists from different fields revealed that a mutated form of a gene called *NOD2* significantly increases a person's risk for developing Crohn's disease. This discovery is built upon research into how genetic and environmental factors combine to initiate an aberrant immune response that cascades into a destructive inflammation of the digestive system.

Crohn's disease typically afflicts young people in their teens and twenties, although it can strike at any age—as President Dwight Eisenhower discovered in his 60's. Symptoms include intestinal inflammation, nutritional deficiencies, abdominal pain, diarrhea, and rectal bleeding. For decades, the only treatment was surgical removal of the affected regions of the intestine. While research has since made possible less drastic alternatives, including oral medication and nutritional supplements, the majority of Crohn's patients still require surgery even today.

A complex interplay of environmental and genetic factors cause Crohn's disease. The environmental component involves the benign bacteria that normally live in our intestines. In healthy people these bacteria do not incite an attack by the immune system, but the immune system of patients with Crohn's disease reacts abnormally against these innocuous bacteria, unleashing destructive inflammation. Evidence for a genetic component comes from research on families in which individuals have the disease. Crohn's disease appears to be genetically complex, involving two or more genes, thus making the hunt for genes involved especially challenging. Research into the genetic and environmental causes of Crohn's disease could lead to the design of novel therapies and new methods for identifying individuals at risk for developing the disease, facilitating early intervention.

Important clues about the genetic and environmental factors underlying Crohn's disease have emerged from studies in animals. In an innovative study, scientists identified a variety of genes in healthy mice that were turned on or off in response to the presence of normal intestinal bacteria. This new knowledge of the response of healthy gut tissue to harmless bacteria may help scientists understand how this response goes awry in Crohn's disease. For insights into the destructive immune response of Crohn's disease, scientists studied mice known as SAMP1/Yit mice, which are genetically predisposed to developing Crohn's-like intestinal inflammation. When these animals are housed in special "germ-free" conditions they remain disease-free, demonstrating that genetic factors alone will not produce the disease. However, in the presence of normal environmental bacteria, SAMP1/Yit mice develop intestinal inflammation that remarkably mimics that of human Crohn's disease. The inflammation in the mice appears to be mediated by immune system cells called "T cells." These cells produce a protein called TNF-α which promotes inflammation. This work also further validates the use of SAMP1/Yit mice as a model of Crohn's disease because a drug used to treat many Crohn's patients, infliximab (Remicade®), also blocks TNF-α activity.

A major advance in unraveling the genetics of Crohn's disease occurred in 1996, when researchers identified a region on human chromosome 16 believed to include Crohn's disease genes. Other scientists, using cutting-edge genetic technology called DNA microarrays, also identified chromosome areas linked to Crohn's disease.

Research took a giant leap forward this past year with the discovery of the first susceptibility gene for Crohn's disease on chromosome 16. The impetus for this discovery was research in another field on an immune gene called *NOD1*. When a draft sequence of the

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human genome was released last year, the scientist who led the team that discovered *NOD1* noticed a very similar gene—*NOD2*—in a region of chromosome 16 previously linked to Crohn's disease. He pointed this out to a colleague who was studying Crohn's disease, and together they used her repository of DNA from 416 families with a history of Crohn's disease to identify a defective form of *NOD2* in about 15 percent of Crohn's patients. The mutated gene also is present in about eight percent of healthy people, indicating that other factors must also interact for the disease to occur. The discovery of *NOD2* mutations in Crohn's disease was validated by a second independent study using a completely different approach, known as positional cloning, to hunt for Crohn's disease genes on chromosome 16. In this second study, additional mutations in *NOD2* were found in Crohn's patients. Having one flawed copy of the gene doubles a person's chances of developing Crohn's; having two copies can increase the risk from 15 to 40 fold. Scientists are now investigating the function of the protein encoded by the *NOD2* gene, and have learned that it activates a molecular factor involved in the response to bacteria. Future studies will attempt to define how mutations in *NOD2* contribute to Crohn's disease.

Extensive research by dedicated scientists and clinicians, coupled with critical advances in technology, have provided the groundwork for extraordinary achievements in genetic research. Scientists studying animal models of Crohn's disease gained insights into the intertwined roles of the immune system of genetically susceptible individuals and naturally-occurring intestinal bacteria in promoting inflammation. The availability of the complete human genome sequence was pivotal in the identification of the first Crohn's disease gene, as was open communication between researchers working in different fields. Finding this gene is a crucial step toward conquering this disease.

PATIENT PROFILE

Rima Matsumoto—Nonalcoholic Steatohepatitis (NASH)

In early 2000, 27-year-old Rima Matsumoto began to think that there was something wrong with her health. She had put on weight and—for no obvious reason—she began feeling tired, lacking energy and stamina. "It wasn't like me," says the upbeat marketing director of a Washington, DC-based nonprofit organization. "I'm a very active person who enjoys playing sports." Indeed, between the ages of 5 and 15 she was an avid and competitive gymnast and weighed about 90 pounds. She had to give that up, however, when she graduated from college. "I became much more sedentary, wasn't eating good food the way I used to, and started to gain weight, but nothing major," says Rima.

What Rima didn't know at the time was that, along with the weight gain, she had developed a liver disease known as nonalcoholic steatohepatitis or NASH. NASH is marked by fat accumulation in and inflammation of the liver. It resembles alcoholic liver disease in many ways, but—as its name implies—it occurs in people who drink little or no alcohol.

After feeling tired and lethargic for six months of 2000, Rima said her health started to affect her personal life. "I didn't want to go out or socialize with friends," she says. "I felt like staying home all the time." She felt depressed and began putting on even more weight. During this period, Rima's liver enzymes, which were being monitored by her doctor, continued to rise—a sign of liver abnormality. By this time, Rima says, she was extremely frustrated. "It was affecting my work and my relationship with my boyfriend." In August 2001, after seeing a specialist and undergoing several more blood tests, Rima was evaluated by the Liver Diseases Section of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). A biopsy of her liver was taken, and it was then that she learned that she had NASH.

About Nonalcoholic Steatohepatitis (NASH)

The underlying causes of NASH are unclear. The condition is most common in adults over the age of 40 who are overweight or have diabetes, insulin resistance, or elevated levels of fat in their blood. In fact, the "typical" patient with NASH is a middle-aged woman who is overweight and diabetic. As Rima's profile shows, however, it is possible for NASH to develop in the presence of only mild weight gain and in the absence of overt diabetes. In such cases, individuals often exhibit an impaired ability to respond to insulin coupled with impaired glucose tolerance (IGT), a dysfunction in the metabolism of the sugar. IGT is particularly insidious because it is often silent and may not be detected until it progresses to overt type 2 diabetes.

Although most people with NASH do not have any symptoms, a great danger of this disease is that it can lead to a cirrhotic liver, a condition in which the liver contains extensive fibrosis that stiffens blood vessels and distorts the internal structure of the liver. Over time NASH can lead to significant scarring of the liver in 30 percent of patients and liver cirrhosis in 10 to 15 percent. Liver cirrhosis is irreversible and may progress to liver failure, ultimately requiring liver transplantation.

Participation in NIDDK Clinical Research

At the time of her referral to NIDDK, Rima weighed approximately 130 pounds. On her 5' 1" frame, this placed her Body Mass Index (BMI) at 25.5: just barely overweight (Note: For a discussion of BMI, how it is used, and other ways to measure body weight, see the sidebar "Who Should Lose Weight?"). She was also insulin resistant, a pre-diabetic condition that often leads to the subsequent development of frank diabetes. Therefore, while Rima does not fit the "classic" profile of a patient with NASH in that she is young and is not diabetic, her condition highlights how NASH can strike beyond its traditional clinical bounds. Even small amounts of extra weight—in the presence of other factors such as insulin resistance and IGT—can predispose some people to NASH. Doctors are increasingly

appreciative of the fact that the disease can occur in persons who are not obese or overtly diabetic and in children as well.

At the NIH Clinical Center, Rima is now enrolled in an NIDDK pilot clinical research study to evaluate the effectiveness of an experimental anti-diabetes medication that acts to improve the sensitivity of the body to insulin. "The clinical trial in which Rima is taking part is a preliminary study. If this medication proves itself to be safe and appears beneficial in improving liver histology, a larger controlled trial will be conducted," says NIDDK research physician Kittichai Promrat, M.D., who is treating Rima for her disease as part of the NIDDK's research efforts.

Rima takes the experimental medication as a capsule once a day, first thing in the morning, and says she is responding well to the medication. Aside from minor headaches, she says she experiences no other side effects and is able to go on with her life as always. "I feel better now," says Rima, "knowing I wasn't making anything up and actually had a diagnosable disease. It relieved a lot of my anxiety."

"My doctors tell me that although I am not diabetic, I am insulin resistant, and that if I don't take care of myself I could become diabetic. They say the most important things for me to do are to watch my diet, lose weight, and exercise."

History of NASH

NASH was recognized as a specific medical condition in 1980. Up to that point, it was considered rare and was referred to simply as fatty liver, fatty hepatitis, or diabetic hepatitis. Today, it is believed that NASH is becoming more and more common in the U.S., most likely as a result of the epidemic increase in obesity. In fact, NASH may affect as many as 5 percent of Americans. It often goes undiagnosed because it causes few symptoms and—when symptoms do occur—they are often vague and non-specific, such as fatigue.

Historically, people with severe cases of NASH were thought to be alcoholics, even when they denied drinking excessively. Since 1980, however, is has become clear that this disease is relatively common and it is not related to drinking alcohol. Rima, for example, says "I don't really drink alcohol except on special occasions or holidays, and only a glass of wine at most."

Current Diagnosis and Treatment Methods

Usually, NASH is initially suspected on the basis of abnormal liver enzymes detected as part of a routine blood test. Further indication of the disease comes from ultrasound examination of the abdomen: a "bright" liver is indicative of elevated levels of fat in the organ. NASH is confirmed if a liver biopsy shows fat, inflammation, and injury. It is important to note that patients with mild elevations in liver enzymes and a bright liver on ultrasound may have no injury to the liver. For these patients, the only way to separate "simple fatty liver" from NASH is by liver biopsy.

Currently, there is no established treatment specific for NASH. Therapies focus on strategies designed to reduce weight and improve the symptoms of diabetes, either through changes in diet and exercise or with drugs. NASH patients who are obese, diabetic and with high lipids in their blood are advised to lose weight and to control their diabetes and elevated lipids. "Many patients benefit just by knowing about the disease and

NEW NIDDK INITIATIVE TO COMBAT NASH

In addition to the pilot clinical study in which Rima is participating, the NIDDK is undertaking a new initiative to establish an interlocking network of cooperative investigators, who will design and implement a database and clinical research network to study the causes, contributing factors, natural history, complications, and therapy of this disease.

working on diet and exercise," says Dr. Promrat. He adds that patients are susceptible to NASH at different levels of obesity, "and some patients, quite frankly, are not obese at all," he adds. Rima's weight, for example, is now 136 pounds, considered slightly overweight for her height, but not obese. She is seeing a dietitian as part of her treatment. She is also aware that there is a history of diabetes on her mother's side of the family. "My doctors tell me that although I am not diabetic, I am insulin resistant, and that if I don't take care of myself I could become diabetic." Thus, although Rima does not fit the typical clinical profile for NASH, her disease may be indicative that the conventional profile is expanding.

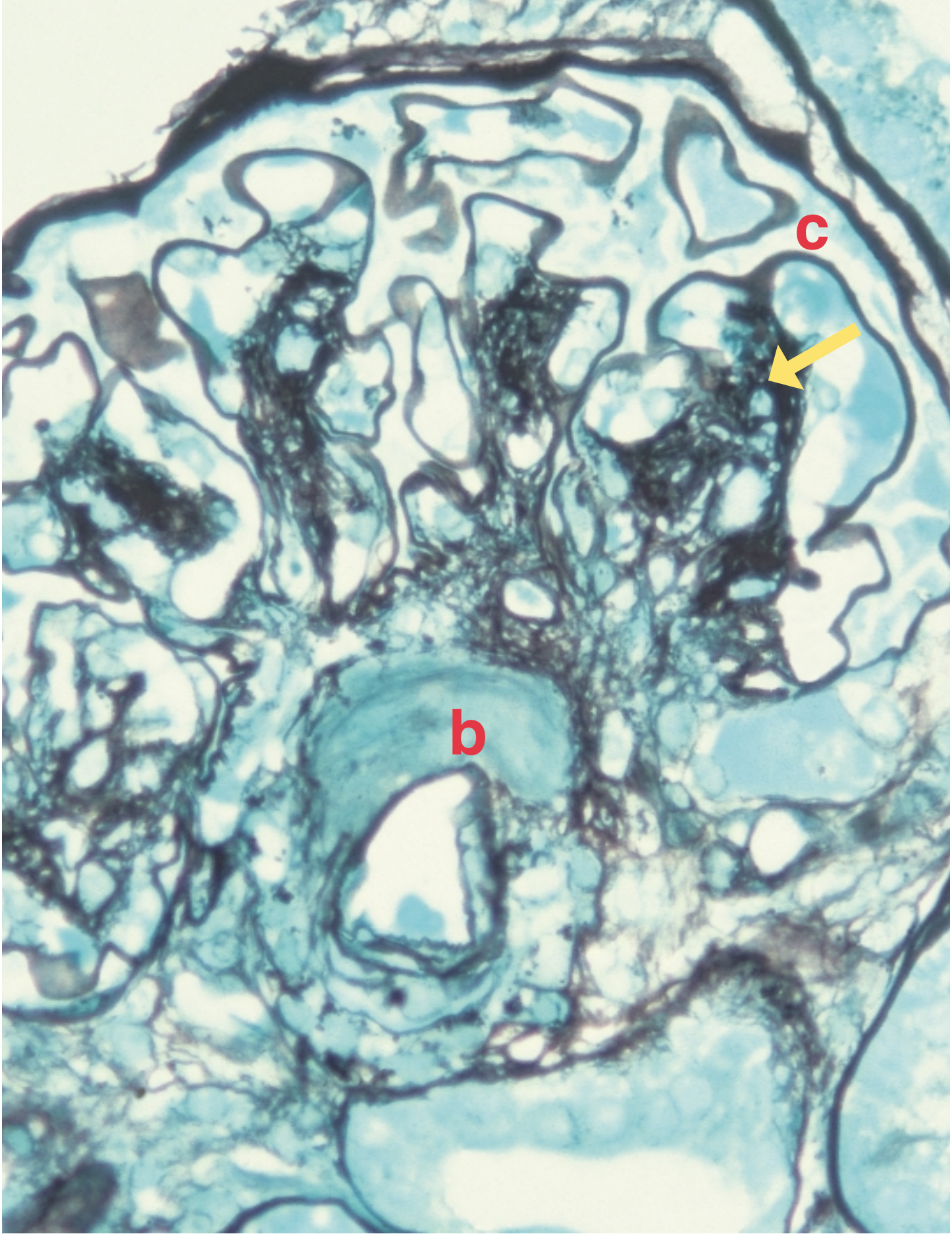
Living with the Disease

Rima says that since she's been in the NIDDK protocol, she has had more energy and—though she says she has her goods days and bad—generally feels much better. "I'm so glad I was recommended to NIDDK and qualified for the protocol," she says. But she's also under no delusions. "My doctor works closely with me, but I know that with my disease I need to do a lot of the work myself, namely watch my diet, lose weight and exercise. I need to take responsibility for my life."

Rima credits a loving support system of family and friends, and especially her boyfriend, with helping her get through the hard times. "If it wasn't for this understanding and support, I think I would've given up even before coming to NIDDK," she says.

NONALCOHOLIC STEATOHEPATITIS (NASH) FACTS

- **As many as 5 percent of Americans may be affected to some degree by NASH.**
- **NASH is marked by accumulation of fat in the liver associated with inflammation and liver cell injury, which, in a proportion of patients, can lead to severe liver damage.**
- **NASH is not connected with other causes of liver disease, including hepatitis B and C viruses, autoimmune disorders, alcohol, drug toxicity, or the accumulation of copper or iron.**
- **There is no known specific cause of NASH and there are no universally agreed-upon treatments for it. Therefore, approaches to treating NASH typically involve drugs to improve the underlying insulin resistance and impaired glucose tolerance as well as changes in diet and exercise to promote weight loss.**
- **NASH can be a silent disease; many affected people are unaware of their condition because they feel well and have no overt clinical symptoms of liver disease.**



This photograph shows a cross-section of a glomerulus, the ball-shaped filtering unit of the kidney, taken from a patient with diabetic kidney disease. The tuft of capillary loops (c) surrounding one of the two central blood vessels (b) found in the glomerulus, has been extensively damaged (dark stain, as indicated by the yellow arrow). Both chronically elevated blood sugar levels and genetic susceptibility appear to contribute to this disease. Photo credit: Dr. Josephine Briggs, NIDDK, and Dr. Paul Killen, University of Michigan.

Kidney, Urologic and Blood Diseases

Diseases of the kidneys, urologic system, and blood are among the most critical health care problems in the U.S. They cause suffering and disability for millions of Americans, including children and young adults. The NIDDK is dedicated to research aimed at understanding, treating and preventing these diseases.

Chronic kidney disease is a growing epidemic in the U.S. It often progresses to irreversible kidney failure, which requires treatment with dialysis or kidney transplantation for patient survival. Presently, as many as 11 million individuals have substantially impaired kidney function. The two main causes of kidney disease, diabetes and hypertension, account for as much as 70 percent of all new cases of chronic kidney disease. The epidemic is due in large part to the increase of type 2 diabetes in the country.

The U.S. has seen an enormous increase in patients with end-stage renal disease (ESRD). In the year 2000, almost 100,000 people entered ESRD, with the result that a total population of about 300,000 were sustained on dialysis and 80,000 with functioning transplants. These numbers have doubled since 1990 and are expected to nearly double again by 2010. The cost of ESRD is high–almost $18 billion in 1999, as well as $2 billion to $4 billion of lost income for patients.

Ethnic minority populations, particularly African Americans, Hispanics, and American Indians, bear a disproportionate burden of chronic kidney disease. African Americans and American Indians are four times more likely and Hispanics are two times more likely to develop kidney failure than are whites.

The NIDDK devotes considerable resources to understanding the basic mechanisms underlying the causes and progression of kidney disease to end-stage kidney failure. The Institute's effort to combat ESRD includes research to reduce morbidity and mortality from bone, blood, nervous system, metabolic, gastrointestinal, cardiovascular, and endocrine abnormalities in ESRD; and to improve the effectiveness of dialysis and transplantation. Major areas of research focus include identification and testing of possible therapeutic interventions to prevent development or halt progression of kidney disease, and identification of the risk factors for ESRD and cardiovascular disease. A major outreach initiative is the National Kidney Disease Education Program.

Genetic Link Discovered for IgA Nephropathy

A principal cause of end-stage renal disease leading to kidney failure is inflammation of the glomeruli, specialized tufts of tiny blood vessels in the kidney that help clean the blood of waste and extra fluid. This inflammatory condition is known as glomerulonephritis. The most common cause of glomerulonephritis is IgA (immunoglobulin A) nephropathy, also called Berger's disease. About 100,000 Americans have IgA nephropathy. The disease may progress over a period of 10 to 20 years. About 30 percent of patients ultimately develop kidney failure. IgA nephropathy usually occurs in adolescents or young adults between the ages of 15 and 35. Males are affected two to three times more frequently than females.

Just why IgA deposits form is not known, although a variety of factors such as genetics and coincident infections seem to play important roles. There also is wide variation in incidence of IgA nephropathy in different parts of the world. IgA protein is a normal part of the body's immune system that protects against disease. In IgA nephropathy, however, IgA protein deposits in the glomeruli. The deposits lead to scarring of the kidneys, which interferes with the blood-cleansing process. The signs of disease are commonly revealed by the presence of blood and protein in the urine. IgA deposits are an immune system defect, hence IgA nephropathy is considered an autoimmune disease of the kidney.

Until recently, there was little reason to believe that IgA nephropathy would have a strong association with a single gene. Now, however, by studying ethnic groups

and families, researchers have located a gene area that is associated with IgA nephropathy. They collected 30 kindreds—24 in Italy and six in the United States—with 94 affected members, all ascertained via biopsy-documented cases. They performed a genome-wide analysis of linkage, searching for chromosome intervals showing linkage to the disease. Surprisingly, they found strong evidence for linkage of IgA nephropathy to chromosome 6q22–23. About 60 percent of kindreds with familial IgA nephropathy have disease attributable to inheritance at this genetic region.

The discovery that IgA nephropathy is influenced by a gene on chromosome 6 has opened the way to better understanding of the cause of IgA nephropathy, and to the possibility that treatment aimed at the molecular cause of IgA nephropathy may one day prevent kidney failure in patients with the disease. Researchers are working to identify the gene itself, a discovery that might yield clues to whether particular environmental influences trigger the disease. For example, the interaction between the gene and an environmental factor, such as an infectious agent, might explain why not everyone who inherits the IgA nephropathy gene develops the disease.

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Genes and Technology Spur Advances in Polycystic Kidney Disease (PKD)

Polycystic kidney disease (PKD) is a genetic disease characterized by massive enlargement of the kidneys, caused by the growth of multiple fluid-filled cysts. It is estimated that PKD affects as many as 500,000 to 600,000 people in the U.S., and is the fourth leading cause of kidney failure.

A landmark advance in PKD research occurred when scientists discovered the genes, known as PKD1 and PKD2, which, when mutated, are responsible for the most prevalent form of PKD. Since discovery of these causative genes, investigators using three mouse models of the disease have made several advances in understanding PKD. They discovered a family of proteins produced by the PKD genes, called polycystins. In the most common form of PKD, the severity of the mutation was directly related to whether the animals died before birth or had decreased life spans. They concluded that the presence of polycystin-2 is essential for normal development of parts of the kidney, heart, and pancreas. A second research team examined kidney cysts from two patients and discovered that 71 percent of the cysts had mutations in the PKD2 gene, while a subset of cysts lacked those mutations but had mutations in the PKD1 gene. The findings suggest that PKD1 mutations may be modifiers of disease severity, and that independent disturbances in the production of the polycystin proteins by the PKD genes may be sufficiently disruptive to cause cyst formation.

Functions of PKD Genes: Before using this knowledge to develop PKD treatments, however, researchers need to know what these genes normally do within the kidney. Recent experiments suggest that polycystin-1 and -2 interact to form a channel, or opening, on the outer surface of cells that permits passage of calcium and other positively charged molecules. In the epithelial cells lining the kidney's filtering tubules, entry of calcium is thought to set off a chain of signals that controls cell growth and promotes normal structure and function of the tubules. Scientists have identified mutant versions of the polycystin-1 and -2 proteins in patients with PKD. One type of mutation prevents polycystin proteins from reaching the cell surface, so they are unable to form a channel. Without the channel, calcium can't enter the cells and signaling is disrupted. Disrupted signaling prevents normal maintenance of epithelial cell growth, and may result in generation of fluid-filled cysts. Another study suggests a second possible mutation that could result in signaling disruption. The end of the polycystin-1 protein that is located on the inside of kidney epithelial cells is called the C-terminus. The C-terminus of polycystin-1 must be intact in order for calcium and other positively-charged molecules to enter kidney epithelial cells. A mutation in PKD-1 that causes loss of the polycystin-1 protein C-terminus would prevent entry of calcium and other positively charged molecules into the cells, again causing signaling disruption and possibly resulting in cyst formation. The vital information provided by these and future studies could pave the way for the development of new and improved approaches for treating polycystic kidney disease.

Use of CAT Scans To Monitor PKD: A crucial accompaniment to research efforts on the causes of PKD is the development of new technologies to assess its progression. Until recently, doctors have had no rigorous guidelines for judging whether or not a PKD patient is likely to develop kidney failure and how quickly the disease may progress. An NIDDK-supported study recently confirmed doctors' anecdotal observations that kidney enlargement due to increased number and size of cysts is an accurate marker of PKD progression to kidney failure. The study used computed tomography (CT) scans to visualize kidney size and monitor the number of kidney cysts over the course of several years. The rate of kidney enlargement varied widely from patient to patient, but it was directly linked to the number or size of kidney cysts. Patients whose kidneys became enlarged were more likely to develop kidney failure, and those whose kidneys remained small were more likely to maintain relatively normal kidney function. This study validated the use of CT scanning as a method for monitoring progression of PKD. Use of CT scanning will also enable doctors to judge how well potential treatments work by documenting whether kidneys and cysts grow, shrink, or remain the same in size. The NIDDK intends to intensify research in this area, in recognition of the enormous value of technologies that can both assess the progression of PKD and also aid in the evaluation of new therapeutic approaches.

Facilitating PKD Research: NIDDK support for PKD research is being strengthened by new work on mouse models and on basic cell biology, in order to understand the cause of disease and to facilitate testing new treatment interventions. Four centers for basic research on PKD have been established by the NIDDK. Another recent NIDDK initiative (Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease, or CRISP) has promoted the development and testing of improved state-of-the-art imaging methods for PKD, including the CT technology described previously; the NIDDK is now expanding its support for both technology development and improved image processing. Under a new initiative, the NIDDK also plans to implement a multi-center clinical trial to assess the best strategy for reducing morbidity and mortality in PKD. The trial will investigate the optimum target levels for blood pressure control for patients with PKD, and whether angiotensin-converting enzyme inhibitors offer superior benefit over other anti-hypertensive agents in slowing the progression of PKD. The hope is that results emerging from studies in these three areas—basic research, technology, and clinical trials of treatment—will complement each other to improve the lives of PKD patients as quickly as possible.

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Kidney Disease and Diabetes

Kidney disease is the major cause of excess illness and premature death in people with type 1 diabetes. Studies have suggested that, although the prolonged high blood glucose levels present in type 1 diabetes play an important role, they do not act alone to cause kidney disease—genetic susceptibility is also required. Recently, NIDDK-supported researchers identified a variation in the apolipoprotein E gene in type 1 diabetics that is associated with a three-times greater risk of developing kidney disease. This association first was found by doing a large, case-controlled clinical study, and was extended to a family-based association study. The latter is perhaps the most reliable method for examining associations between DNA sequence differences and specific diseases. While several studies have yielded equivocal results about the association of this genetic variant with kidney disease, this study is perhaps the most definitive to date. The apolipoprotein E gene codes for a protein that plays an important role in cholesterol transport. The particular variant of the gene that the researchers identified is strongly

associated with the development of coronary artery disease. One important next step for this research is to determine the molecular mechanisms that underlie the risk for diabetic kidney disease that is caused by the apolipoprotein E gene variant.

Recent advances in genetic technology have made it theoretically possible to generate mice that will develop diabetic complications analogous to human diabetic kidney disease. To facilitate studies in this area, the NIDDK is currently sponsoring a research program, the Mouse Models of Diabetes Complications Consortium. The Consortium is generating genetic mouse models to analyze the initiation and progression of diabetic complications, including kidney disease. Such accurate models of human diabetic kidney disease, once developed, will be especially valuable in uncovering the genes and cellular processes that confer susceptibility or provide resistance. Candidate methods for the prevention, detection, and treatment of diabetic kidney disease may also be effectively tested in these mouse models. Other related research programs sponsored by NIDDK include a clinical trials pilot program to identify the most promising interventions for preventing or slowing the progression of kidney disease in type 1 diabetic patients.

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Preventing Kidney Failure

Kidney disease exacts a heavy toll on the U.S. population, in terms of disease and death. To overcome this burden on the public's health and well-being, the NIDDK has developed and carried out a number of initiatives to identify the most appropriate treatments for kidney disease, the risk factors associated with complications, and the best ways to increase the public awareness of kidney disease, in order to prevent progression to irreversible kidney failure.

African American Study of Kidney Disease and Hypertension (AASK): African Americans constitute approximately 12 percent of the U.S. population but comprise 32 percent of the prevalent ESRD population. In African Americans, the racial disparity is most striking in younger people, where those between the ages of 25 and 44 are 20 times more likely to develop kidney failure caused by high blood pressure, or hypertension, than whites. While better management of hypertension has led to fewer strokes and heart disease, kidney failure is increasing.

This past year, significant progress was made in identifying a treatment strategy for slowing the progression of kidney disease caused by hypertension. In 1994, the NIDDK began the largest U.S. clinical trial of kidney disease in African Americans, called the African American Study of Kidney Disease and Hypertension (AASK). It was hoped that this trial would determine whether doctors should treat patients with elevated blood pressure to a level lower than is usually practiced, whether a specific class of blood pressure drug is required, or whether both strategies are needed to slow or stop the progression of hypertension-related kidney disease in African Americans. Scientists working on this study recently showed that people with kidney disease caused by hypertension have a better chance of reducing the risk of kidney failure if they take an angiotensin-converting enzyme (ACE) inhibitor. They found that the ACE inhibitor ramipril slowed kidney disease by 36 percent and slashed the risk of kidney failure and death by 48 percent in patients who had at least one gram of protein in the urine. The drug was compared to the dihydropyridine calcium channel blocker amlodipine. Results were not related to blood pressure control, which was comparable between study groups.

ACE inhibitors have been the preferred treatment for kidney disease caused by diabetes since 1994. Now, AASK doctors are recommending it for kidney disease of hypertension, especially for people who also have protein in the urine. While calcium channel blockers help many patients, particularly African Americans, control blood pressure and reduce the risk of stroke and heart disease, patients may need an ACE inhibitor to protect the kidneys.

When the AASK trial concludes in 2002, the NIDDK will support investigations of the environmental, socioeconomic, genetic, physiologic, and other co-morbid factors that influence progression of kidney disease as part of the AASK Continuation Study.

Cohort Study of Chronic Renal Insufficiency (CRIC): The NIDDK is launching a major new clinical initiative to increase understanding of the risk factors associated with

progression of kidney disease and the development of cardiovascular disease and associated mortality. This initiative recognizes that cardiovascular disease is the leading cause of death in patients with ESRD, and that it is imperative to gain new knowledge about the relationship between ESRD and cardiovascular disease as a foundation for the development and evaluation of potential interventions. To this end, the NIDDK is initiating the "Cohort Study of Chronic Renal Insufficiency (CRIC)."

CRIC is a prospective longitudinal study that will examine genetic, environmental, behavioral, nutritional, quality of life, and health resource utilization factors in people with chronic kidney disease. CRIC also will determine the incidence of and risk factors for cardiovascular disease in these individuals. The participants in this study will reflect the racial, ethnic, and gender composition of the end-stage kidney disease patient population in the U.S., including an appropriate number of African Americans.

National Kidney Disease Education Program (NKDEP): Chronic kidney disease can be prevented in many populations at risk. Moreover, its progression can be slowed in those who already have the disease, as shown in the AASK trial and other studies. Despite these advances in treatment and prevention, data suggest that only a small fraction of people at serious risk for or with established but early kidney disease is receiving proper screening or treatment. To remedy this problem, the NIDDK recently initiated the National Kidney Disease Education Program (NKDEP), which will address the epidemic of kidney disease in the United States (see sidebar on page 66).

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Understanding the Function, Structure, and Genetics of the Urinary Bladder

Through research, scientists are discovering that the urinary bladder is much more than a reservoir for liquid waste. Rather, it is a dynamic organ with many important structural and physiological properties. As the kidneys filter waste from the blood, they send it to the urinary bladder for collection as urine. When the bladder is full, nerve impulses sent to the brain signal that it is time to urinate, resulting in the sensation of "urgency." Proper function of the bladder is vital to rid the body of waste and to prevent infection. Unfortunately, millions of Americans suffer from disorders affecting bladder function, including temporary problems with urine retention; chronic and painful disorders such as interstitial cystitis, which can cause scarring and permanent bladder damage; urinary tract infections, which can move up through the bladder to endanger the kidneys; and urinary incontinence, resulting in uncontrolled urine flow from the bladder. Recent findings from NIDDK-supported research on bladder genes and proteins are providing insights into normal bladder function that could, in turn, lead to better tests, treatments, and prevention strategies for bladder disease.

Protecting the Bladder's Permeability Barrier: Recent studies have elucidated the functional importance of a class of four proteins, called uroplakins, found only in the urinary tract. Plaques of crystalline uroplakin particles almost entirely cover the bladder lining (urothelium). They are vital in the permeability barrier that protects the bladder from infectious agents and prevents leakage of waste products into the body. The plaques are also fairly dynamic structures that can break and re-fuse, which may be important in maintaining barrier flexibility as the bladder distends and retracts during filling and emptying.

Upon examining the interactions of the uroplakin proteins in animals, NIDDK-supported investigators found that two of the four proteins link specifically to the other two (uroplakin UPIa with UPII, and uroplakin UPIb with UPIII). The two pairs are present in all plaques, and all plaques have a similar uroplakin composition. It also appears that both uroplakin pairs are required for normal plaque formation, for when the same group of researchers removed the gene for uroplakin III in a mouse model, the bladder lining became permeable.

Upon examining the remaining uroplakins, the researchers found several abnormalities in UPIII's partner protein, UPIb. The end result was that only small patches of urothelial plaque were formed, causing the permeability defect. Just as importantly, knocking out UPIII also caused the mouth of the tubes (ureters) connecting the kidneys to the bladder to become larger, resulting in vesicoureteral reflux, in which urine flows back from the bladder into the kidneys. In humans, vesicoureteral reflux is a hereditary condition that affects about one percent of pregnancies and represents a leading cause of renal failure in infants. Until now, there was no known genetic basis for this disease. Although reflux is likely caused by more than one gene, these new data now suggest that the absence of uroplakin III can cause this defect, opening up avenues for future therapies.

Dynamic Role of Bladder in Releasing Proteins into the Urine: Further dismantling the image of the bladder as primarily a "receptive" organ, NIDDK-supported researchers have also found that the bladder can secrete a number of different proteins that go directly into the urine rather than forming part of the bladder lining. Depending upon the animal, proteins previously identified in the urine come either directly from the kidneys, or from the liver *via* the kidneys. The proteins secreted into the urine by the bladder itself, which include both enzymes and enzyme inhibitors, may have important physiological or protective functions in the lower urinary tract. Importantly, this finding also suggests that the bladder may play a more dynamic role in responding to its environment. For example, if bladder proteins are inappropriately secreted in response to factors encountered in the urine, this could act as a trigger for bladder dysfunction.

These new findings are providing great momentum to bladder research at the cellular level. By exploiting these discoveries, scientists can propel bladder research even further to reveal, with even greater precision, how the bladder functions, and how bladder diseases can be optimally treated and prevented.

In addition to supporting basic and applied research in bladder disease, the NIDDK seeks to enhance progress in bladder disease research efforts by engaging in strategic research planning with scientific leaders in the external research community. Consistent with this goal, the NIDDK convened the Bladder Research Progress Review Group (Bladder Research PRG) meeting in the summer of 2001. This group was composed of representatives from professional and patient organizations and experts in specific bladder diseases from a broad range of research disciplines. These experts evaluated the bladder research portfolios of NIDDK and NIH, identified research opportunities, and defined unmet needs in bladder research. A strategic plan to redefine bladder research and to target specific areas for expansion is being formulated by the Bladder Research PRG. The report from this meeting will be invaluable in guiding future NIDDK efforts in bladder disease research.

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Bladder Cancer Diagnosed by Simple Urine Test

NIDDK-supported researchers recently developed a urine test that could lead to an easier, less invasive way to detect bladder cancer. This new test has enormous therapeutic implications, as it may help patients avoid partial or total loss of the bladder to cancer, which can affect quality of life, even beyond the inability to urinate normally. In both men and women, the proximity of internal sexual organs to the bladder means that they may have to be removed in cases of invasive bladder cancer. As with all cancers, early detection of bladder cancer is vital to avoid such drastic surgery. Bladder cancer is the fourth most common type of cancer in men and the eighth most common in women. Right now, there is no simple, approved test for bladder cancer.

The simple, noninvasive test screens for a protein called survivin, which is undetectable in most normal adult tissues, but is prominently expressed in common human cancers. Survivin is an inhibitor of programmed, or "natural," cell death. When the survivin gene is switched on, subsequent production of survivin permits mutated cells to survive. Switching off the survivin gene

stops the progression of cancer. The new test uses an antibody to detect survivin in urine samples.

In a series of experiments, survivin was found in all (46) urine samples of patients with new or recurrent bladder cancer, but not in the urine of any of the healthy volunteers (17) or patients with other urologic cancers, i.e., kidney, prostate, cervical, or vaginal cancer (30). The results of these and other experiments indicate that the sensitivity of the urine survivin test for new or recurrent bladder cancer was 100 percent, and its specificity for other noncancerous and benign genitourinary tract disease was 95 percent. The researchers suggest that their simple, noninvasive, urine survivin antibody test would be a useful complement to other diagnostic markers to identify new bladder cancers early, to monitor bladder cancer patients more effectively, and to identify recurrences early. With this impressive research advance, it is hoped that a urine test for bladder cancer could become as routine as tests used at other regular check-ups, such as prostate specific antigen (PSA) tests for prostate cancer.

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Urinary Tract Infections: Insights into Causes and Treatments

Urinary tract infections (UTIs) are among the most common infectious diseases acquired by humans; in fact, only respiratory infections occur more often. Women are especially prone to UTIs, in part, it is believed, because women have shorter urethras than men. Urine flows out from the bladder through the urethra during urination, and this "tube" is the primary site of UTIs. Most UTI's arise from one type of bacteria, *Escherichia coli (E. coli)*, that normally lives in the colon. If the infection is not treated promptly, bacteria may travel to the bladder, which can lead to a relatively serious infection of the kidneys, a condition known as pyelonephritis. UTIs are treated with antibacterial drugs. The choice of drug and length of treatment depend on the patient's history and the urine tests that identify the offending bacteria and its relative sensitivity to various drugs.

Asymptomatic UTIs: Scientists and physicians have begun to realize that often, a woman may have bacteria in her urine but not have symptoms of a UTI. These types of infections are called "asymptomatic" because the patient does not display any physical signs of a UTI. A recent study demonstrated that asypmtomatic infections are relatively common and rarely persist for a long period of time. Thus, bacteria in the urine do not automatically lead to clinically overt UTIs. However, such asymptomatic infections are strong predictors of subsequent, symptomatic UTIs.

Recurrent UTIs: Many women suffer from frequent UTIs. Nearly 20 percent of women who have a UTI will have another, and 30 percent of those will have yet another. Of the last group, 80 percent will have recurrences. Usually, the latest infection stems from a strain or type of bacteria that is different from the infection before it, indicating a separate infection. Even when several UTIs in a row are due to *E. coli*, slight differences in the bacteria indicate distinct infections.

Because UTIs are a recurrent problem for a large number of women, there has been interest over the years in determining whether it may be beneficial for women to self-diagnose and self-medicate with antibiotics as a valid approach to treating these chronic, recurrent infections. In order to determine the effectiveness of this strategy, researchers studied 172 women in a university-based primary health care clinic. Within this group, 88 women diagnosed a total of 172 UTIs, about 94 percent of which were subsequently confirmed by laboratory evaluation, and treated themselves with the antibiotics ofloxacin or levofloxacin. Self-treatment of uncomplicated recurrent UTIs was very effective in curing infection in this study, as the cure rate exceeded 90 percent.

While self-diagnosis followed by self-treatment simplifies the care of women with recurrent UTIs, it will nevertheless still be important to involve physicians and other health care professionals in the management of these infections, especially in light of the growing problem of drug-resistant bacteria, described below.

Antibiotic Resistance: The use of antibiotics to combat UTIs needs to be tempered with caution in selecting the appropriate drug. Because some strains of bacteria are resistant to certain drugs, it is important to choose agents that will be effective against a given strain of *E. coli*. For example, a recent study showed that empirical treatment of UTIs with trimethoprim-sulfamethoxazole (TMP-SMX),

initiated before the results of microbiological tests were known, led to lower cure rates in individuals who were subsequently found to be infected with organisms resistant to this drug.

In many cases, the current drug of choice for treatment of uncomplicated UTIs is TMP-SMX, though many other antibiotics are used. However, the frequency of UTIs caused by bacteria that are resistant to TMP-SMX is increasing. The widespread overuse of antibiotic drugs has led to increased incidence of multiple-drug resistance in *E. coli*, including those strains that can cause UTIs. The appearance of antibiotic-resistant bacteria is worrisome because it eliminates that drug as a treatment option. Multiple-drug resistance, therefore, severely limits the efficacy of existing antibiotics to treat infection.

Responding to a sharp increase in drug-resistant UTIs, researchers recently identified a new strain of *E. coli*, which they called clonal group A, in urine samples from women with UTIs in California, Michigan, and Minnesota. In this study, scientists examined *E. coli* isolated from young women in a university community in California who had uncomplicated urinary tract infections that were resistant to TMP-SMX. The results of the analyses were compared with bacteria isolated and analyzed from similar patients in Michigan and Minnesota. The bacteria isolated from these patients were tested for a number of molecular markers and for antibiotic susceptibility. In the California group, over one half of the urine samples tested positive for *E. coli*, and nearly one-quarter of these consisted of bacteria that were resistant to TMP-SMX as well as other antibiotics. Half of the antibiotic-resistant samples of *E. coli* displayed a common DNA fingerprint, suggesting that the bacteria isolates belonged to the same genetic strain, dubbed "Clonal group A" by the investigators. Similar findings were noted in the analysis of patients from Michigan and Minnesota. This prevalence of Clonal group A *E. coli* is surprisingly high for a single strain. Importantly, the distinct geographic clusters of the patient groups studied suggest a common route of dissemination of Clonal group A, possibly through contaminated food. This finding indicates that molecular typing of the *E. coli* causing drug-resistant UTIs may provide important information about the origins and spread of these bacteria within communities, enhancing opportunities to prevent further transmission of drug-resistant infections.

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Prostate Disease: New Clinical Efforts

Diseases of the prostate, including benign prostatic hyperplasia (BPH) and chronic prostatitis, are a major health care burden for men. BPH affects more than 50 percent of men past the age of 50. If left untreated, BPH can lead to urinary tract infections, urinary retention, and in occasional cases, kidney disease. Chronic prostatitis is a chronic, disabling condition in which pelvic pain is the most common symptom. Such pain is followed by various voiding symptoms, such as waking up at night to urinate; frequent, urgent urination; incomplete voiding; decreased force or intermittency of the urinary stream; and/or a need to push or strain to fully empty the bladder. Because these diseases of the prostrate represent a potential burden to men of all ages and ethnic backgrounds, the NIDDK has undertaken vigorous efforts to study these problems, to identify their underlying causes and search for new potential therapies.

Developing Biologic Markers and Genetic Tests: The NIDDK is seeking better biologic markers for BPH, as well as genetic tests to identify patients at high risk for early disease, for rapidly progressing disease, and for prostate cancer. Genetic tests could also be used to determine which patients are likely to respond to various forms of therapy. It is likely that such important tools for aiding diagnosis and treatment may be developed from biologic materials that have been accumulated as part of the NIDDK's ongoing clinical trial, the "Medical Therapy of Prostatic Symptoms" (MTOPS). Nearing completion, the MTOPS trial is testing two different

drug regimens for BPH. One drug, finasteride, inhibits formation of a hormone involved in prostate enlargement, while the other, doxazosin, relaxes the muscle of the prostate and bladder neck to improve urine flow and reduce obstructions.

New biologic and genetic tools may be developed by further study of the serum samples of the 4,000 patients who participated in the MTOPS clinical trial, as well as the tissue from prostate biopsies of approximately 900 patients. The availability of these biologic materials presents an extraordinary opportunity to develop and evaluate markers that will further our understanding of fundamental processes contributing to BPH and prostate cancer, or related to response to therapy of BPH. The large number of well-characterized patients in the MTOPS consortium should enable the identification and testing of such biomarkers. To take advantage of this valuable resource, the NIDDK intends to assemble a cross-disciplinary, multi-institutional consortium with a range of expertise to perform cooperative studies. This consortium will use the MTOPS material to evaluate genetic, immunologic, or biochemical biomarkers relevant to the progression of BPH, response to therapy, and the concurrent development of prostate cancer.

Another recent and complementary NIDDK initiative will expand the cadre of prostate researchers and increase the use of novel technologies and innovative approaches in prostate research as part of the Institute's Prostate Research Novel Exploratory Teams (Prostate Research NET). This initiative is the result of several information-gathering and planning meetings conducted in coordination with other NIH institutes and centers, including the National Cancer Institute-sponsored Prostate Research Progress Review Group, April 1997; the International Symposium on Prostate Growth, March 1998; and the Symposium on Prostate Growth and Aging, September 2000.

Assessing New, "Minimally Invasive" Surgical Treatments: Research funded by the NIDDK has contributed to the development of new "minimally invasive" surgical treatments for BPH, which will now be assessed for long-term safety and efficacy. For many years, transurethral resection of the prostate (TURP) has been the standard of surgical therapy for treatment of symptomatic BPH. With TURP, an instrument called a resectoscope is inserted up the urethra through the penis. The resectoscope has a wire loop at its end that the physician uses to remove the tissue obstructing the urethra. Transurethral procedures are less traumatic than open forms of surgery and require a shorter recovery period, but are not ideal.

Technical innovations over the past decade have furthered the development of more advantageous surgical treatments. These new "minimally invasive" surgical treatments aim to achieve the same long-term outcomes of TURP, but with the benefits of lower costs, shorter length of hospital stay, and more rapid recovery. These approaches include laser therapy, transurethral electrovaporization, microwave therapy, and transurethral needle ablation.

To assess the quality of outcomes of these new therapies, the NIDDK has formed a group of collaborative Prostate Evaluation and Treatment Centers and a Biostatistical Coordinating Center. These centers will develop and conduct randomized, controlled clinical trials of the long-term efficacy and safety of the major "minimally-invasive" approaches for the treatment of symptomatic BPH. Through carefully designed and controlled clinical trials, a clearer picture of the benefits and risks of these methods will become available, thus aiding the physician and patient in making the most appropriate treatment choices.

Chronic Prostatitis Collaborative Research Network: The NIDDK's Chronic Prostatitis Collaborative Research Network has developed and validated a questionnaire being used by the wider research community to provide accurate assessments of symptom severity and quality of life. The Network is documenting symptoms, possible risk factors, medical histories, treatments, and the results of blood, prostate fluid, semen, and urine tests. Three avenues of study are being pursued through the Network: basic laboratory investigation, a longitudinal cohort study, and a randomized clinical trial. The Network recently began its first clinical study by comparing the effects of two drugs with placebo. The drugs are: (1) tamsulosin hydrochloride, which may increase the flow of urine and decrease pelvic pain, and (2) ciprofloxacin, an antibiotic that may reduce inflammation. Two additional clinical sites have been added to facilitate recruitment of minority study participants, and full-scale implementation of trials with all sites recruiting patients is slated to occur in 2002.

Stem Cell Approaches for Blood Disorders

Sickle cell anemia is an inherited, chronic blood disease in which the red blood cells become altered and function abnormally. The disease is caused by a change in the chemical composition of the protein, hemoglobin, which carries the oxygen inside red blood cells. This abnormal hemoglobin, called "hemoglobin S," causes the shape of the molecules to change under certain conditions and chemically link to each other, creating chains of molecules called polymers. Elongated hemoglobin S polymer structures distort the shape of the whole red blood cell, which interferes with red blood cell movement through blood vessels. Damage results to the vessels around the distorted cells and the tissues that depend on the vessels for oxygen and nourishment. Sickle cell anemia can become life-threatening when the red blood cells break down or the bone marrow fails to produce blood cells during periodic sickle cell "crises." Repeated episodes such as these can lead to damage of the kidneys, lungs, bone, liver, and central nervous system. Sickle cell disease is a serious disease in the African American community.

The abnormal hemoglobin S is inherited as an autosomal recessive trait; this means that it must be inherited from both parents for a person to contract sickle cell anemia. If hemoglobin S is inherited from only one parent, the offspring will "carry" the sickle cell trait, but are usually without symptoms. No cure is available for sickle cell anemia and the current objective of therapy is the comprehensive management and control of symptoms relating to sickle cell crises. However, even with treatment of symptoms, patients with sickle cell anemia usually die from organ failure between the ages of 20 and 40. New research in therapies for sickle cell anemia capitalizes on the molecular basis for the disease, which makes it a good target for genetic correction at the stem cell level. Current research is under way in the NIDDK intramural program to pursue stem cell-based approaches to this disease, successful completion of which would eliminate all symptoms and complications of patients with sickle cell anemia. Researchers in the NIDDK's Molecular and Clinical Hematology Branch are currently investigating stem cell-based approaches to sickle cell anemia; two of these approaches are allogeneic bone marrow transplantation and autologous stem cell gene therapy.

Allogeneic Bone Marrow Transplantation: The first approach under study is stem cell replacement by allogeneic bone marrow transplantation, in which the donor, either a relative or an unrelated individual from a registry, is genetically similar to the patient. Bone marrow contains the stem cells responsible for producing red blood cells and transplantation has become an accepted curative therapy for a broad range of diseases, including malignant diseases such as leukemias and lymphomas, as well as non-malignant and inherited diseases such as sickle cell anemia.

For effective transplantation, high doses of chemotherapy and/or radiation must be given to the patient in order to destroy the defective bone marrow and suppress the recipient's immune system to decrease the chance of graft rejection. This process is called myeloablative conditioning. The normal marrow obtained from the donor is delivered intravenously to the patient and the stem cells find their way to the bone marrow to produce new, normal blood cells. This ablative conditioning, however, can be toxic to the patient, causing significant acute side effects including hair loss, vomiting, disease of the heart muscle, and acute kidney failure. NIDDK researchers are now aiming to develop a nonmyeloablative conditioning regimen in which both the donor's and the patient's stem cells would contribute to normal blood formation. In this scenario, although the patient's defective stem cells would be present, the normal donor stem cells should have a selective advantage over the defective ones, thus favoring normal red blood cell formation.

Using a non-myeloablative conditioning regimen, NIDDK researchers have shown that bone marrow transplants into mice with sickle cell anemia result in complete replacement by donor hemoglobin, reflecting the selective advantage conferred upon the normal hemoglobin-containing red blood cells. With this success, plans are under way at NIDDK to pursue a non-myeloablative bone marrow transplant clinical trial for patients with sickle cell anemia.

Autologous Stem Cell Gene Therapy Approaches: A second type of stem cell-based correction is also being pursued because many sickle cell patients lack a suitable matched donor for bone marrow transplantation and even those with a donor are at risk of developing graft-versus-host disease (GVHD). About half the patients undergoing bone marrow transplants develop GVHD, which results

when the donor's bone marrow attacks the patient's organs and tissue. This attack happens because the donor's T-lymphocytes, a type of white blood cell, recognize the patient as being foreign. In most cases GVHD is mild, but it can be life-threatening. For these patients, autologous stem cell gene therapy may be useful. It is a process by which the patient's own stem cells are removed from the bone marrow, genetically corrected, and then reinfused into the patient to contribute to normal red blood cell formation and permanent correction of the sickle cell defect. Gene transfer generally involves the identification of a therapeutic gene or other nucleic acid (for example, an RNA molecule or a synthetic nucleic acid piece), a vector that allows delivery of the therapeutic nucleic acid to the appropriate cell, and a device (for example, a catheter, syringe, or stent) to deliver the gene/vector combination to the appropriate tissue *in vivo*.

Gene transfer can also be achieved through the use of a modified retrovirus or adenovirus vector to enhance gene delivery to certain cells. Using a modified retroviral vector, researchers have demonstrated gene transfer rates of greater than 10 percent in the rhesus monkey, a primate with stem cell biology similar to humans. This is the first time results such as these have been seen in a large animal and they mark a major advance for the field. Also, persistent, successful engraftment was seen in the nonhuman primates after less toxic, low dose irradiation. The NIDDK intramural research team continues efforts to refine and optimize techniques for gene delivery. They have begun to develop a preclinical nonhuman primate model for gene transfer, which could soon become a viable form of therapy for patients with sickle cell disease.

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The National Kidney Disease Education Program (NKDEP)

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health has recently initiated the National Kidney Disease Education Program (NKDEP). This program will raise awareness about the seriousness of kidney disease, and the importance of prevention, early diagnosis, and appropriate management of the disease and its complications.

More than eight million Americans have seriously reduced kidney function, and nearly 400,000 require dialysis or a kidney transplant to stay alive. The number of people developing kidney failure has doubled each decade for the last two decades, and will likely continue to do so. These increases appear to parallel the rising prevalence of diabetes, the leading cause of kidney disease.

Fortunately, kidney failure can be slowed, if not prevented. Evidence shows that blood glucose and blood pressure control can reduce the risk of kidney complications. Low protein diets have also been shown consistently to lessen progression as well. Despite these advances in treatment and prevention, only a small fraction of people at serious risk or with established but early kidney disease are receiving proper screening or treatment.

The program is being modeled after the successful federal National High Blood Pressure and National Diabetes Education Programs. The NIDDK held a series of planning meetings between July 2000 and June 2001 to review the current state of kidney disease in the United States and to develop strategies for the education program. A Steering Committee of kidney organizations, federal agencies, community organizations and health professional organizations was convened in September 2001 to provide guidance in developing the program's priorities.

The program will target primary care providers and people at high risk for kidney disease, particularly those with diabetes, hypertension and/or a family history of kidney failure. In its first phase, NKDEP will conduct educational campaigns for at-risk African Americans and health care providers in four pilot sites. Messages will focus on identifying risk factors for kidney disease, screening those at risk, and providing appropriate treatment for those who are diagnosed with kidney disease. The four pilot sites are Baltimore, MD; Atlanta, GA; Jackson, MS; and Cleveland, OH. These sites will enable the program to identify successful strategies to launch a broader national campaign. In its next phase, NKDEP will broaden its reach to American Indians and Hispanic/Latinos.

Other program activities include creating a compendium of existing educational programs and resources on chronic kidney disease; developing clinical tools for primary care providers; improving laboratory reporting of kidney function; and developing an evaluation plan to assess the program's impact on the target audiences at the pilot sites as well as the long-term impact of the overall program.

STORY OF DISCOVERY

Bacterial Pili—Molecular Initiators of Bladder and Kidney Infections

It all starts with a "handshake" between two molecules of almost infinitesimally small size: a tiny, hairlike projection from the surface of a bacterial cell and a small cluster of sugar molecules on the surface of a human epithelial cell. From this molecular interaction arise over eight million doctor visits each year. A urinary tract infection (UTI)—which may involve the bladder, kidneys, or both—begins when a molecule on the surface of a bacterial cell recognizes and binds to a molecule on the surface of its target urinary tract epithelial cell. Bladder and kidney infections are important public health concerns because many individuals suffer recurrent infections. Because of the wide prevalence of UTIs, their tendency to recur, and the potential for bladder infections to progress to more serious kidney infections, researchers have long sought to understand the first critical steps in infection. The identification and characterization of the mechanism by which bacteria adhere to epithelial cells is the key to designing therapies to block this process and prevent these infections. Recently, research from the field of structural biology—the study of how molecules interact with one another—has yielded important insights into the earliest stages of UTIs and provided fresh evidence that strategies to block the attachment of bacteria may represent an effective way of treating the initial infection as well as a viable approach to preventing their recurrence.

The adhesion of bacteria to the cells of the urinary tract is mediated by proteins present on the surface of the bacterial cells. Over twenty years ago, studies implicated these proteins in this process. Since that time, families of proteins have been discovered, the genes that encode them have been cloned, and they have been the subject of much research. Although the genetics of these bacteria are well-characterized, studies of bacterial genetics illustrate a paradox about modern molecular biology: as more is learned about the genes of organisms, the more researchers appreciate that genes tell only part of the story. A gene represents a set of instructions for assembling a chain of amino acids in a specific sequence to form a protein. In order for this to occur, a gene must first be turned "on" so that the information within the gene can be read by the cell. This blueprint must then be faithfully translated into a mature protein. However, functional proteins do not exist as linear chains of amino acids; rather, they fold back upon themselves, assuming intricate shapes with highly complex topography. To truly understand how a protein functions, and how it interacts with other proteins, it is often necessary to understand how these proteins are assembled from their genetic instructions, how this process is regulated, and what form these proteins ultimately assume.

Among the people looking for this knowledge are structural biologists—scientists interested in how molecules come together to form critical components of cells. These researchers have long studied protein assembly using bacteria as a model system. Pili are tiny rod-shaped projections from the surface of many bacteria that can act as adhesion molecules, facilitating cell-to-cell contact and communication. Structural biologists have determined that these hairlike pili are formed through a complex series of interactions that begin within assembly of the pilus base within the bacterial cell and the transport of the elongated pilus across the outer cell membrane. As a model system to study protein assembly, pili have proven to be a very rich source of information about the mechanics of protein assembly and transport within the cell. But pili are important for reasons beyond their value as models of protein assembly.

In UTIs, attachment of bacteria to the surface of the epithelial cells that line the urinary tract is a key event, and a better understanding of this process might reveal novel approaches to preventing infection. Not surprisingly, this initial interaction is mediated in large part through proteins on the surface of the bacterium that

recognize and bind specifically to molecules on the surface of the epithelial cells—including pili. Pili consist of multiple protein subunits each encoded by a specific gene. It has long been known that the presence of certain kinds of pili on a cell's surface increases the number of *E. coli* capable of infecting the urinary tract and enhances the persistence of the infection. Although all strains of uropathogenic *E. coli* possess the genes necessary to give rise to pili, the expression of a particularly critical one is controlled by a small segment of DNA located nearby. This small invertible element is capable of "flipping" its relative orientation within the chromosome and thereby controlling gene expression: when it faces one direction, expression of the gene is turned "on" and pili are present on the cell surface; conversely, when the element faces the opposite direction, the gene is turned "off" and the protein is absent. Relatively little has been known about why this element flips, how its orientation might change during an actual infection, and what the implications of these changes could be.

To answer these questions, scientists recently isolated different strains of *E. coli* from cystitis—bladder infection—or pyelonephritis—kidney infection—and then examined the ability of the bacteria to change the orientation of this invertible element. After culturing both bacterial strains under conditions that maintained the element in the "off" position, the strains were introduced into mice. Subsequently, the bladders were removed and the orientation of the invertible element within the bacteria analyzed. The element in the bacterial strain that was originally isolated from the cystitic strain quickly reverted to the "on" orientation; in contrast, it remained largely "off" in bacteria from the pyelonephritis-causing strain. This observation suggests that cystitis-causing strains have an enhanced ability, relative to pyelonephritis-causing strains, to change the orientation of the invertible element to the "on" position and thereby alter pilus gene expression. This finding suggests that bacteria that infect the bladder may rely more heavily on pilus-mediated attachment during infection than bacteria that infect the kidney. It also illustrates how changes in expression of a particular gene—and not its presence or absence—can influence the site of infection in UTIs.

Once bacteria find themselves in the kidney, pili again play a role in adhesion and infection. A protein that sits atop the pilus—PapG—is involved in recognizing a specific molecule on the surface of the kidney cell and thereby mediating binding of the bacteria to the kidney cell. PapG's partner in this molecular interaction is a group of sugar residues on the outer membrane of the human kidney cell—globoside. Scientists have deduced the three-dimensional structure of PapG alone and in a complex with globoside. This detailed molecular snapshot—with a resolution finer than one-millionth of a meter—has allowed researchers to identify which areas of the PapG protein mediate binding of the bacterium to the host cells, as well as to pinpoint which regions of the host globoside are important for this interaction. By defining the specific molecular interaction necessary for binding to occur, this insight not only sheds light on a key event in infection but may also lead to the development of vaccines that target the disease process at its earliest stages.

Even with all of these insights into the molecular events that underlie the earliest stages of infection, the sad fact remains that, after a successful course of antibiotic therapy, a significant number of women will suffer a relapse of their UTI. Why this is so has remained a mystery for many years. New research has provided fresh insights into why many bladder infections recur—and once again highlights the role of pili. It seems that bacteria use their pili not only to recognize and bind to bladder cells during the initial infection but also to get inside of these cells. Invasion of these bladder cells may provide the bacteria with a relatively safe environment in which they may either replicate or go into "hibernation," only to emerge later—and cause a subsequent UTI. According to this model, following the initial UTI the bacteria persist at low levels, continuously invading a small number of cells, escaping, and re-infecting neighboring cells. Insights into the role of pili in this process might allow the design of therapies designed to interrupt this cycle of infection and relieve the burden of recurrent UTIs.

STORY OF DISCOVERY

The study of pili illustrates how technical and highly specialized research into a seemingly obscure question—how proteins are assembled in bacteria and what shapes they assume—can also have relevance to a common and costly health issue—UTIs. From bacterial models of protein assembly to critical players in infection and possible targets for future therapies, the story of pili shows that there is no such thing as something too small to investigate.

PATIENT PROFILE

Art and Pat Joseph—New Immunosuppressant Approaches to Kidney Transplantation

Husband and wife Art and Pat Joseph are a perfect match in more ways than one. In 1990, at age 26, Art was diagnosed with a serious kidney disease, called focal segmental glomerulosclerosis, or FSGS. FSGS describes the regional scarring or hardening of a portion of the million or so tiny clusters of looping blood vessels, or glomeruli, that serve as filters within the kidneys. These glomeruli are essential for processing about 400 quarts of blood a day to sift out an estimated two quarts of waste products and extra water that eventually leave the body as urine. FSGS hampers the filtering process. The bottom line is that most patients with the disease progress to kidney failure, often referred to as end-stage renal disease (ESRD), within five to 20 years after diagnosis. In Art's case, 11 years passed from the time he was diagnosed until the time he was in desperate need of a kidney transplant. But thanks to a loving wife able to give one of her kidneys to her husband, as well as NIDDK-sponsored scientific research that has led to advances in immunosuppressant drug therapies to prevent rejection of transplanted organs, this is a story with a happy ending.

Art Joseph (right) was diagnosed with serious kidney disease in 1990. He successfully received a donor kidney from his wife Pat two years ago, under a newly developed transplantation protocol. The two are now counselors for other patients considering the procedure, and look forward to "traveling and growing old together," as Pat says.

The Signs and Symptoms of Glomerular Disease

There are several warning signs that can signal glomerular disease, including:

- Large amounts of protein in the urine (proteinuria), which may cause foamy urine.
- Blood in the urine (hematuria), which may cause urine to be pink or cola-colored.
- Inefficient filtering of waste from the blood.
- Low blood protein (hypoproteinemia).
- Swelling in parts of the body (edema), including hands and ankles, especially at the end of the day, or around the eyes when awakening in the morning.

Often, however, these symptoms go undetected. Such was Art's case. Had it not been for a medical exam while he was stationed in Japan with the U.S. Navy, the then Petty Officer Second Class may never have learned that he had FSGS until years later. "They discovered large amounts of protein in my urine," says Art. A biopsy of his kidneys led to a confirmed diagnosis of FSGS. To decrease the protein in his urine and improve his kidney function, Art was placed on a cortisone-like steroid to reduce kidney inflammation. However, steroids can promote insulin resistance in a significant number of patients. Six weeks after being put on steroids Art was in a near coma in a Scottish hospital, the result of steroid-induced type 2 diabetes. "I

was feeling real bad, so my wife made me go to the doctors," he says. A glucose test indicated Art's sugar level to be off the charts—856 (normal blood sugar levels are between 60 and 110 if an individual is fasting, and between 80 and 120 following a meal). He was immediately taken off steroids and given daily injections of insulin to control his sugar level. Within weeks his glucose started to come back into the normal range, and within six months he no longer needed to take insulin. For a while, life for Art returned to almost normal.

Diagnosing Kidney Disease

Urinalysis provides information about kidney damage by indicating levels of protein and red blood cells in the urine. Blood tests measure the levels of waste products such as creatinine, a waste product in the blood that results from the normal breakdown of muscle (healthy kidneys filter creatinine from the blood), and urea nitrogen to determine whether the filtering capacity of the kidneys is impaired. If these lab tests indicate kidney damage, a doctor also may recommend ultrasound or an x-ray to see whether the shape or size of the kidneys is abnormal.

Living with the Disease

As kidney function becomes impaired, most people with a glomerular disease, including FSGS, lack energy, feel listless, and lose their appetites. Many must periodically take time off from work. Fortunately for Art, aside from regularly scheduled exams to monitor his kidney function and having to take medication to control his blood pressure, he was able to go for years without any fundamental lifestyle changes. "I continued to work, play football, basketball, lift weights, ride my bike," he says. "My doctors were amazed that I was able to sustain such a high activity level for so long." But over time, the disease started to take its toll. In 1994, Art's creatinine level started to creep up, and by 1999, it was obvious that Art would require a kidney transplant within a year. Once again, Art was extremely fortunate. When they learned of his need, about 20 of his friends and relatives said they would be willing to donate one of their kidneys to him. As fate would have it, tests showed that a kidney taken from Art's wife, Pat, would be the best option. Despite the risks, including a slightly increased risk of high blood pressure or kidney failure of her own, Pat says, "I was delighted to learn that mine was the best match, and I never gave donating one of my kidneys to Art a second thought."

Facts About Kidney Disease and Transplants

In recent years, National Basketball Association superstars Alonzo Mourning of the Miami Heat, and Sean Elliot of the San Antonio Spurs (retired) have raised awareness of kidney disease as a result of their being diagnosed with focal segmental glomerulosclerosis (FSGS). The facts are:

- More than 50,000 Americans die each year because of kidney disease.
- More than 260,000 Americans suffer from chronic kidney failure, and to stay alive require either an artificial kidney machine (dialysis) or a kidney transplant.
- More than 35,000 patients are waiting for kidney transplants, but only about 11,000 will receive them this year because of a shortage of suitable organ donors.

TYPES OF KIDNEY DISEASE

Glomerular diseases—including glomerulonephritis (inflammation of the membranes in the kidney), and glomerulosclerosis (scarring or hardening of the tiny blood vessels within the kidney)—interfere with the kidneys' ability to filter body fluids. Focal segmental glomerulosclerosis (FSGS) refers to a regional glomerulosclerosis of the kidney. All can lead to kidney failure, or end-stage renal disease (ESRD). These diseases may be the direct result of an infection or a drug toxic to the kidneys, or may result from diseases that affect the entire body, like autoimmune diseases such as type 1 diabetes or lupus.

New Experimental Protocol

But Art and Pat Joseph were faced with yet another major decision. Once transplanted with his wife's kidney, Art could decide to either go on a standard therapy of immunosuppressant drugs, which includes steroids, to prevent his body from rejecting the new organ or undergo a new protocol developed by NIDDK researchers. "Because I already had had an episode of steroid-induced type 2 diabetes, if I chose the standard therapy I ran a fifty-fifty chance of becoming a life-long diabetic," says Art. He and Pat talked it over. Another major consideration was the fact that he would be only the second person to undergo the new protocol. They decided on the new protocol. Art's case was immediately transferred from Walter Reed Army Hospital to NIDDK. Tests showed that he was a good candidate for the new protocol, and his transplant took place February 8, 2000.

Art was treated using a new approach to kidney transplantation. Researchers at NIDDK have hypothesized that one of the main reasons that people reject a kidney transplant is that the immune system is activated by the surgical trauma necessary to put the new kidney in. Since the immune system has evolved to protect people from infection at the time of injury, it is likely that there are trauma-associated triggers that spur on the immune system to seek out foreign tissue. Thus, if the immune system could be sequestered until the trauma from the surgery was healed, NIDDK researchers postulate that the rejection response will be greatly reduced and controlled with a minimal amount of immunosuppression, hopefully avoiding the need for steroids and other harmful drugs.

As part of a new treatment protocol, Art received an experimental drug called Campath-1H. This drug temporarily removes T cells (the cells that cause organ rejection). Rather than putting Art on multiple anti-rejection medications from the start, he was given no medication, watched closely, and placed on the amount of immunosuppression necessary only when the Campath effect wore off. For Art, that was a single daily dose of a liquid called sirolimus. He did not need steroids or cyclosporine. As this document goes to press, Art is two years from his transplant and has had no signs of rejection. He has required about 15 percent of the immunosuppressive drugs that he would have received under standard therapy. Importantly, given his history, he has not required any steroids.

Today, Art, who received a medical discharge from the U.S. Navy in 1996 and now works at the Office of Naval Intelligence in Suitland, Maryland, says that he is "back to normal." Other than having experienced a slight fever immediately after the first dose of the drug protocol, he says he has had no other side effects. "The only kidney medication I take is a liquid called sirolimus, which I drink each morning with orange juice to slow down the activity of my white blood cells," says Art. "I went back to work three weeks after my transplant. I could have gone back in two, but my wife and mother didn't want me to," he adds.

Art and Pat now volunteer as counselors for other patients who are considering undergoing the new protocol for organ transplantation. "We were treated wonderfully by everyone at NIDDK," says Pat, who adds that her operation to donate her kidney went flawlessly. "They answered whatever questions we had and were available to Art and me all hours of the day and night. Counseling others is our way to give something back." Since the transplant, Pat, who will be retiring from the Navy in a few years, says she's more protective of and feels a closer bond to her husband. "I can now look forward to our traveling and growing old together," she adds happily.

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